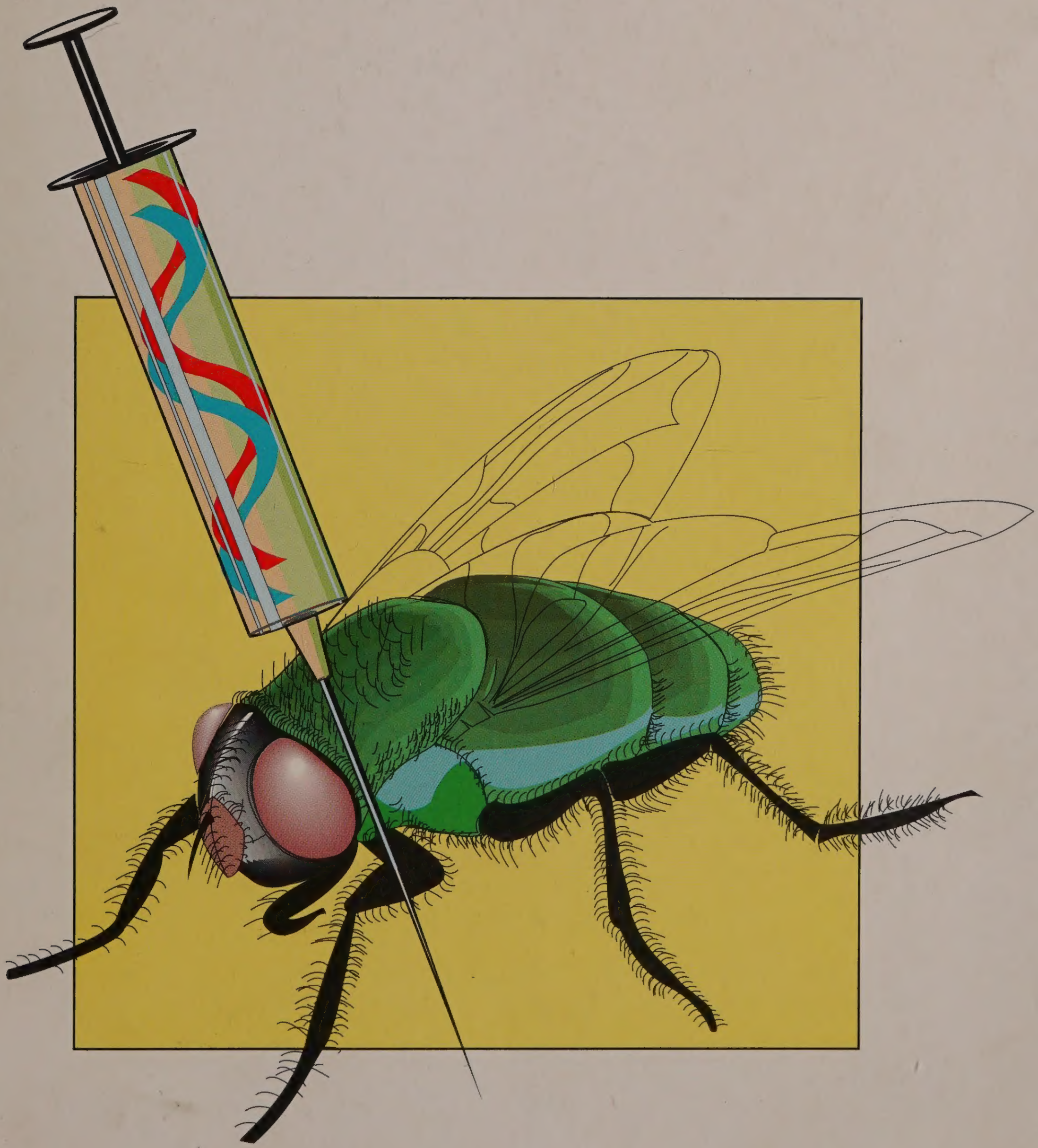
DIRECTORATE FOR NATURE MANAGEMENT

Ecological risks of releasing genetically modified organisms into the natural environment

DN report 1991 - 7b



Cover illustration: Knut Kringstad

22501548821

Ecological risks of releasing genetically modified organisms into the natural environment

DN report 1991 - 7b

INFORMATION CENTRE
28 JUL 1993 3984
Wellcome Centre for Medical Science

TRONDHEIM

Directorate for Nature Management
Tungasletta 2, N-7005 Trondheim
Telephone + 47 7 58 05 00 - Telefax + 47 7 91 54 33

DN report

1991 – 7b

TITLE Ecological risks of releasing genetically modified organisms into the natural environment	DATE Nov. 1992
PERSONS RESPONSIBLE/AUTHORS A committee appointed by the Directorate for Nature Management	NUMBER OF PAGES 55
DEPARTMENT/SECTION Department of Freshwater Ecology	SIGNATORY Peter J. Schei Peter Johan Schei Director of DN

ABSTRACT

At the request of the Directorate for Nature Management (DN) the committee looks at the relationship between modern biotechnology and the external environment. Emphasis is placed on the predictable outcome of deliberately or unintentionally releasing genetically modified organisms in the near future. The risks and potential ecological impacts of undertaking such releases into the natural environment are assessed. The report also reviews the research and development requirements that need attending to before good assessments of potential environmental impacts of intended releases can be adequately provided. One conclusion drawn by the committee is that risk assessments should follow the internationally established practice whereby each case is assessed individually (case-by-case) using a stepwise procedure (step-by-step) based on the worst conceivable course.

DN will, in due course, consider the recommendations of the committee and undertake an overall evaluation of the relationship between biotechnology and the environment.

3 STIKKORD PÅ NORSK	3 KEY WORDS IN ENGLISH
Bioteknologi	Biotechnology
Genetisk modifiserte organismer	Genetically modified organisms
Økologisk risiko	Ecological risks

FOREWORD

Background for appointing a committee of experts

The environmental authorities have already considered the first application for the deliberate release of genetically modified organisms (GMO's) and more applications for experimental releases are likely to need assessing soon; applications for commercial releases will come in due course, too. To meet this situation, there is a need for more knowledge about the possible effects of these releases on the environment.

Genetically modified micro-organisms, plants and animals represent great potentials for both the primary sector and biotechnologically-oriented industry. The number of genetically modified systems will therefore increase in the future, both as regards the number of species undergoing gene transfer and the number of properties affected in this manner. As the development of molecular biology proceeds, new techniques for transferring genes can be expected to be evolved, and increasingly more complex genetical relationships will be the objective of such operations. In this connection, the environmental authorities will be faced with many new challenges.

The Directorate for Nature Management (DN) in Norway has therefore taken an initiative to obtain better understanding of which problems are likely to arise when genetically modified organisms are released into the natural environment. To ensure a well-founded scientific assessment, DN has appointed a group of specialists.

The members of the committee

professor	Odd Halvorsen	University of Oslo
professor	Terje Traavik	University of Tromsø
research officer	Kjetil Hindar	NINA
research officer	Morten Laake	NIVA
research officer	Reidar Mehl	SIFF
research officer	Odd Arne Rognli	NLH
senior engineer	Guri Tveito	SFT
senior executive officer	Heidi Bente Hoel Draget	DN

Professor Odd Halvorsen chaired the committee. Yngve Svarte (DN) substituted for Heidi Bente Hoel Draget during her leave of absence. Jan Husby (DN) acted as secretary.

The mandate of the committee

"It is intended that the committee shall specify important problem areas in the relationship between biotechnology and the natural environment and examine these in the context of the sphere of responsibility of environmental management and the challenges this has to face. The work carried out by the committee will form the scientific basis for a strategy to be formulated by the environmental authorities.

The committee shall:

- identify and map important problems arising from the deliberate or unintentional release of GMO's (genetically modified organisms) into the natural environment
- assess the need for research and development to improve the background knowledge needed to evaluate the risks involved in releasing GMO's into the natural environment
- formulate proposals for the scope and content of consequence reports relating to the release of GMO's into the environment; a consequence report in this context embraces consequence analysis, prior investigation and follow-up investigation
- provide recommendations that will form the basis for building up a data base, especially as regards which data should be stored; the data base will ensure that the environmental management and research sectors have easy access to knowledge about GMO's in the natural environment.

The committee must prepare a report provisionally entitled:

Ecological risks of spreading genetically modified species in the environment".

Within the time limit set, the committee shall be free to choose its working methods and the priority it places on the various tasks".

The deliberations

Eight meetings have been held in connection with the work and with visits to laboratories involved in genetic engineering in Norway. A workshop has also been arranged where invited scientists evaluated the report and discussed important issues highlighted by it. The committee would like to thank the participants for their useful comments and fruitful discussions and trusts that this product is in line with those discussions. The programme and a list of the participants are enclosed with the report. The committee would like to thank Øyvind Bakke (NINA) and Knut Kringstad (DN) for their contributions to the report.

This English-language version of the original report in Norwegian (DN-rapport 1991-7) has been translated by Richard Binns, M.Sc., Trondheim.

A "DN-Notat" (no. 10, 1991) has also been published which contains enclosures to this report (in Norwegian) in which viruses, plants and biological control are dealt with in greater detail. The respective enclosures have been written by Terje Traavik, Odd Arne Rognli and Reidar Mehl.

On behalf of the committee

Odd Halvørsen

CONTENTS

1. INTRODUCTION 9

1.1 What are genetically modified organisms (GMO's)? 9
1.2 Future trends - what will the next 10 years bring? 10

1.2.1 Viruses 12
1.2.2 Micro-organisms 12
1.2.3 Algae, fungi and higher plants 13
1.2.4 Animals 14
1.2.5 Expected developments in Norway 15

2. POSSIBLE ECOLOGICAL EFFECTS 17

2.1 Potential risks of introducing genetically modified organisms 17
2.2 Comparison with introduced species 24

3. ASSESSING THE RISK OF UNDESIRABLE ENVIRONMENTAL IMPACT 27

3.1 Two principles when assessing risks 27
3.2 Use of mathematical models in risk assessments 30
3.3 Criteria for risk assessments 31
3.4 Special features of environments for deliberate release in Norway 35

4. REGULATING MODERN BIOTECHNOLOGY 36

4.1 International regulation 36
4.2 Regulation and management in Norway 39

5. NEED FOR RESEARCH AND DEVELOPMENT 41

6. CONCLUSIONS 43

7. GLOSSARY 45

8. REFERENCES 50

9. ENCLOSURE 55

1. INTRODUCTION

Modern biotechnology utilises organisms that are produced or modified with the aid of cell and/or gene technology. Cell technology permits cells from different organisms to be fused; gene technology enables the genetic material and traits of living organisms to be altered systematically and genes to be transferred between various types of organism.

Modern biotechnology marks a fundamental advance in that it offers greater opportunities for transgressing species boundaries. Gene technology, in particular, provides means for constructing new, recombinant organisms and interfering with and shaping the hereditary characteristics of organisms in ways which were hitherto inconceivable. Deliberate and unintentional releases of new organisms of this kind into the natural environment raise a number of ecological, safety and management questions which this report clarifies.

1.1 What are genetically modified organisms (GMO's)?

The committee defines a genetically modified (transgenic) organism as an organism whose genetic material has been modified with the aid of cellular and/or genetical engineering methods. This definition corresponds with the definitions used in NOU (1990:1) and the Norwegian White Papers on biotechnology (Stortingsmeldinger Nos. 8 and 36, 1990-91).

Use of the following techniques is considered to give rise to genetically modified organisms:

- cloning and transferring genes using vectors (transformation)
- direct injection of genetic material
- fusion of two or more cells using techniques that do not occur naturally.

Use of the following techniques is not considered to give rise to genetically modified organisms:

- *in vitro* fertilisation (test-tube insemination)
- induction of polyploidy (more than two of each chromosome)
- mutations induced by, for example, radioactive radiation
- cell or protoplast fusions with species which are able to cross naturally.

The USA uses a more restricted definition of a GMO than most European countries in that it does not look upon any products of cell or protoplast fusions as being genetically modified, even when such fusions take place between species that do not cross naturally.

1.2 Future trends - what will the next 10 years bring?

By the end of 1990 there were reports of a total of 246 deliberate releases of genetically modified organisms globally (Williamson *et al.* 1990). Most of these are cultivated plants and bacteria that have been supplied with marker genes and/or genes to provide enhanced resistance to disease, pests and herbicides (Table 1). The number of deliberate releases is expected to increase substantially. Worldwide during 1991, there have probably been around 2000 field trials using transgenic plants.

Deliberate or unintentional releases of genetically modified organisms into the environment are likely to occur in connection with agriculture, industry, aquaculture, biological treatment of pollutants and the use and disposal of human and veterinary medicines (Table 2).

Table 1. *Reported deliberate releases of genetically modified organisms and viruses by the end of 1990 (after Williamson et al. 1990)*

	USA	Europe	Other places	Total
Types of released organisms:				
Viruses	7	17	3	27
Micro-organisms	41	8	4	53
Plants	60	41	28	129
Animals	1	0	1	2
Total	109	66	36	211
Types of genetical modification:				
Markers	22	35	10	67
Herbicide resistance	25	18	15	58
Insect resistance	35	10	1	46
Vaccines	7	5	6	18
Virus resistance	10	2	1	13
Cold tolerance	7	1	1	9
Bacterium resistance	1	0	1	2
Others	17	12	4	33
Total	124	83	39	246

Table 2. *Some genetically modified organisms that have been deliberately released or may be deliberately released in the future (adapted from OTA, 1988)*

MICRO-ORGANISMS

Bacteria as pesticides: "Ice-minus" bacteria to reduce frost damage to crop plants.

Bacteria as carriers of *Bacillus thuringiensis* toxin to reduce insect damage to corn crops.

Mycorrhizal fungi to increase plant growth rates by improving efficiency of root uptake of nutrients.

Plant symbionts: Nitrogen-fixing bacteria to increase the nitrogen available to plants and reduce the use of artificial fertilisers.

Treatment of toxic waste: genetically modified bacteria to degrade toxins in waste water, sewage, muds, soil, rubbish dumps, etc.

Recovery of heavy metals: genetically modified bacteria to recover metals from mines and low-grade ore (e.g. copper and cobalt).

Pollution control: possible use of bacteria to remove phosphorus, ammonia, etc. from drinking water.

Viruses as pesticides: host-specific insect viruses with enhanced virulence against insect pests in agriculture (e.g. Baculo-viruses).

Mixoma virus modified to enhance its virulence against rabbits (which became resistant during earlier biocontrol efforts in Australia).

Viruses as vaccines against human diseases: hepatitis A and B, polio, herpes, AIDS, rabies and respiratory syncitial virus.

Vaccines against animal diseases: pseudorabies in pigs, rotavirus in pigs, vesicular stomatitis, foot and mouth disease, rotavirus in cattle, rabies, foot rot in sheep, infectious bronchitis virus (chickens), erythoblastosis in birds and sindbis virus (sheep, cattle, chickens).

Multivalent vaccines: vaccines that are active against antigenetically complex infections, e.g. those causing malaria, sleeping sickness and schistosomiasis.

PLANTS

Plants resistant or tolerant to herbicides: Glyphosate, Atrazine, Sulphonylurea, Imidazolinone, Bromoxynil and Phosphinotricin.

Disease resistance to bacteria and viruses: crown gall disease (tobacco), tobacco mosaic (and related viruses) and potato leaf roll virus.

Pest resistance: plants protected against *Bacillus thuringiensis* toxin. Seeds with enhanced "anti-feedant" content to reduce losses through insect attack during storage.

Enhanced stress tolerance to salt, drought, temperature and heavy metals, etc.

Introduced nitrogen-fixation: nitrogen-fixation in other species than leguminous plants, independent of symbiotic bacteria.

Plants with altered nutritional composition, e.g. fat, starch and protein.

Genetically modified marine algae: algae with enhanced production of substances such as B-carotene and agar, or to enhance ability to sequester heavy metals (e.g. gold and cobalt) from sea water.

ANIMALS

Genetically modified domestic animals to enhance growth and product quality, improve reproductive performance, enhance resistance to disease and improve coat quality, etc.

Genetically modified domestic animals as producers of medicines and other mammalian pharmaceutical products.

Fish with enhanced growth rate, cold tolerance and disease resistance, for use in aquaculture.

1.2.1 Viruses

Viruses have a potential for controlling pests, as vaccines and as vectors for transferring genetic material to animals and humans. The vaccinia virus is used today as a vector for foreign genes. Recombinant vaccinia virus can function as a living vaccine against rabies, hepatitis B and, in principle, any infectious disease. Attempts are now being made to construct a vaccinia virus containing several foreign genes, intended for use as multivalent, living vaccines. Vaccines can sometimes be constructed by cloning genes which provide protective immune response without the disease-producing genes. This is impossible in other cases, but a gene can then be mutated in such a way that it retains the immunological effect, but loses the disease effect. By using these methods it will be possible to circumvent some of the problems at present associated with the use of what are known as weakened, live virus vaccines. Large-scale field trials to combat rabies by using recombinant vaccinia-based vaccines embedded in fox bait have already been performed in France and Belgium.

Baculoviruses have been proposed as alternatives to chemical insecticides. Many insects that are agricultural and forestry pests are members of the Lepidoptera (butterflies and moths) or Hymenoptera (wasps, bees, ants) orders. Some species in these orders are natural hosts for baculoviruses, and such viruses are now being developed that are more effective but less capable of surviving in the wild. To achieve this, genes coding for specific hormones, enzymes or toxins are inserted into the virus genome, whilst an attempt is made to modify or eliminate the viral genes that ensure survival and dispersal.

1.2.2 Micro-organisms

Bacteria, yeast and mould fungi are relatively easily modified by genetical engineering. Many ways of utilising genetically modified micro-organisms are now being developed. In the bacterium *Pseudomonas syringae*, which normally lives on the leaf surfaces of plants, the gene for a protein in the cell membrane that forms nuclei for ice-crystal formation has been removed. Spraying plants with "ice-minus" bacteria of this kind can help reduce frost damage to strawberries and potatoes, for example. Phytophagous insect larvae can be controlled by transferring genes for the formation of toxins from *Bacillus thuringiensis* (Bt toxin) to bacteria which grow elsewhere, e.g. *Clavibacter* sp. which live inside the xylem of plants. It is hoped that genetical modification of bacteria living on the surface of root hairs on maize will provide protection against root-eating insect larvae.

Other bacteria are genetically modified to enable them to degrade specific pollutants in industrial effluent, solid waste, sewage plant silt and contaminated soil and groundwater. Many micro-organisms can do this naturally, but it is feasible to enhance their ability to degrade oil products, solvents and benzene derivatives, perhaps also halogenated hydrocarbons such as PCB's and dioxins. The use of gene technology in this context lies some years into the future.

Considerable interest centres around attempts to improve, or transfer, the ability of micro-organisms to fix atmospheric nitrogen into biologically usable, bound nitrogen. This can help to reduce the need for artificial fertilisers in agriculture, thereby reducing the problem of nitrates contaminating water supplies. Field trials are at present being performed using a number of bacteria that have high nitrogen-fixing ability, but numerous genes are involved and interaction with other processes in the cells is difficult to control. The most ambitious aim, transferring the ability for nitrogen fixation directly to the cultivated plants themselves, will probably take many years to realise.

Blue-green algae rank with bacteria as regards their potential in gene technology, both for industry, agriculture and nutrition. However, since they require a great deal of light they often need to be grown in open systems which approach deliberate release into the environment. It may be possible in the future to use genetically modified blue-green algae in the production of unsaturated fatty acids in dietetical foods, natural colouratives for fishfeed and foodstuffs, protein-rich fishfeed, antibiotics and medicines.

1.2.3 Algae, fungi and higher plants

Myceloid and mould fungi have been used for centuries in production contexts on a level with micro-organisms. Their use as nutrients and feeds must really be regarded as open systems and the environment can, for example, be exposed to large quantities of antibiotic-producing mould fungus. Genetically engineered strains will gain ground by improving taste properties, the nutritional value of feeds, productivity, etc. In industry, genetically manipulated mould fungus is used to produce enzymes and antibiotics, and treatment of waste from these processes is problematical because it is difficult to prevent accidental releases.

Genes coding for resistance against herbicides such as glyphosate, sulphonylurea and atrazine have been introduced into crop plants by genetic transformation to limit loss of yield. These herbicides can therefore be used on a larger scale without being detrimental to the crop plants. Depending on the herbicide concerned, this practice can either increase or decrease the risk of chemical contamination of the environment through a change in the pattern of use.

Plants are also changed to improve their resistance to disease. Resistance to crown gall disease has been achieved in tobacco, on which experiments can easily be performed. Tobacco and tomatoes have also been "vaccinated" against tobacco mosaic virus by adding a single virus gene to the plant genome. Corresponding results with clover mosaic virus suggest that the same method can protect a number of plants from virus attack.

Work is being carried out on several other applications, such as drought tolerance, high salt content in the soil and low temperatures, to adapt plants to areas that are marginal for cultivation. Gene splicing and cell fusion are used to create new varieties of plants, improve the nutritional value of some species and increase the production of additives from certain micro-algae for use in foodstuffs. The future may perhaps show algae being used to sequester valuable metals from sea water, and seeds being enhanced to produce substances protecting them against insect attack, thereby significantly improving their storage qualities.

1.2.4 Animals

In the animal kingdom, the development of gene technology is being directed towards farm animals and fish, with a view to improving reproduction, raising growth rates and feed utilisation, increasing resistance to disease and improving other quality-related characteristics. This is entirely in line with the development that has taken place using traditional breeding methods. However, new elements have been added, such as transfer of growth hormone genes from mammals to fish. It must, nonetheless, be said that in the case of warm-blooded mammals and fish a great deal has to be done before side effects caused by disturbances to the normal functioning of the genetic apparatus are under control. The use of domestic animals such as sheep to produce foreign proteins expressed in their milk is the most far-reaching development.

Experiments with regard to fish involve transferring genes which will increase their weight, tolerance to cold, or resistance to disease. This transfer most frequently takes the form of micro-injection of genes from other species, for example a gene for cold tolerance from a flatfish species to salmon. An experiment carried out in America has involved injecting carp with growth-hormone genes from rainbow trout before releasing them into ponds.

It will be possible to use gene technology to breed such insects as Chinese silkworm moths *(Bombyx mori)* and honey bees *(Apis mellifera)*. Gene technology also seems to offer possibilities for transforming insect pests and insects that transmit disease into harmless variants, a process that may be useful in biological control. Deliberate releases of sterile (not genetically modified) males of the Codling Moth, Mediterranean Fruit Fly, Screw Worm Fly and malaria-carrying mosquitoes have been successfully performed. Large numbers of sterile males with destroyed chromosomes have been released into a specific area to out-compete fertile males during mating. Reproduction dropped substantially and the species will be controllable within the area. The same combative strategy is now being used in experiments with genetically modified insects. Blow-flies *(Lucilia cuprina)* released on the Furneaux Islands between Australia and Tasmania in 1990-91 were genetically modified in such a way that most of the males produced were sterile and the females blind.

The industrial production of parasitical nematodes and insects such as ichneumon wasps, and predators such as green lacewings, ladybirds and rove mites, has become important in many countries. Gene technology will predictably obtain a footing in these fields now.

1.2.5 Expected developments in Norway

The first applications to release GMO's will probably come from the agricultural and aquacultural sectors, and in connection with the biological destruction of pollutants. More industrial application of gene technology can also be expected in Norway. Use in human and veterinary medicine is also likely to increase. This trend may represent a danger for unintentional releases of GMO's into the natural environment.

Genetically modified plants and animals developed in Norway will probably not reach the market this century. More applications for experimental releases of transgenic plants are to be expected in Norway. A private firm has submitted the first application for deliberate release, and the scope of the experiments will partly depend on the extent of private-sector activity. The authorities will probably have to handle applications to test transgenic varieties of plants from abroad submitted to the official plant-testing system in Norway. It is difficult to estimate the numbers of such applications, but since activity has advanced much further internationally than in Norway, there may well be more foreign applications than Norwegian ones.

Genetical engineering has advanced farthest in the case of plants. Table 3 gives an overview of what is known to be taking place in this field in Norway. The table shows that this mainly involves research. The only project having the clearly defined objective of changing an economically important trait is the introduction of virus resistance into Cock's-Foot Grass (*Dactylus glomerata*), run by the Norwegian Plant Protection Institute (SPV) and the Agricultural University of Norway (NLH). However, the deliberate release of plants from this project will not take place in the relatively near future. The only application that has been handled in Norway regarding the deliberate release of potatoes concerns testing of marker genes and not changes in economically important traits in the plant. Transgenic plants have already become important research tools in fundamental studies on gene regulation and gene expression. This may be the field in which transgenic plants will achieve their greatest importance. Since little of this research involves creating fully developed individual plants, there is no question of their planned introduction into the natural environment. However, as a step in the study of genetic stability in different environments, applications for the deliberate release of transgenic plants can be expected.

Industrial exploitation of genetically modified types of plants with changed contents of sugar, starch, fatty acids or fibre has great potential, but lies far into the future.

Considerable media interest was created in Norway some years ago when human growth-hormone genes were micro-injected into salmon and rainbow trout eggs. The experiments showed that the inserted gene was "read", but stopped before it became clear whether the gene had become incorporated into the genome of the salmon. The research group carrying out the trials stopped the experiment that used salmon in 1986 and is now working on genetical modification of a carp, the zebra danio, and a killifish, the medaka (Peter Alestrøm, NLH, Ås, pers. comm.).

Another Norwegian research group isolated growth-hormone genes from salmon and micro-injected them into salmon eggs producing 10 transgenic salmon which were crossed and slaughtered in autumn 1990. Their progenies are now being studied to see whether the genes have been inherited (Audun Nerland, Bergen, pers. comm.). The Norwegian Council for Fishery Research is funding a new experiment in which salmon eggs have been injected with a modified growth-hormone gene from salmon in order to study heredity. The firm that originally funded the experiments no longer has any commercial interest in them. Interest for producing transgenic fish for aquaculture may, however, increase in the future.

Considerable research and development work on gene technology is being carried out in Norway relating to lactic acid bacteria used in dairy products and for conserving fodder. This research is both privately and publicly funded. Genetically modified organisms will most likely be brought into use in this field during the present decade.

Genetically modified micro-organisms to combat diseases and genetically modified "living" vaccines will probably come during the 1990's. In these areas, we will be faced with import of products to a far greater extent than will be the case with transgenic plants and animals.

Table 3. *Overview of work on genetically modified plants in Norway*

Institution	Plant species	Property
Unigen/Inst. of Botany, UNIT *	Birch	Marker gene
	Begonia	– " –
	Lentil	Hormone sensitivity
	Carrot	– " –
	Potato	Marker gene/hormone sensitivity
	Rape	– " –
NLVF*/NLH*	Tobacco	Marker gene
NLVF/NLH	Rice	– " –
	Maize	– " –
	Barley	– " –
SPV*/NLH	Cock's-foot grass	Virus resistance
NISK*/NLVF	Spruce	Chitinase

* UNIT - University of Trondheim, NLVF - Agricultural Research Council of Norway, NLH - Agricultural University of Norway, SPV - Norwegian Plant Protection Institute, NISK - Norwegian Forest Research Institute.

In addition, the Norwegian Plant Protection Institute is doing work on cloning of chitinase genes with a view to combating fungi and insects.

2. POSSIBLE ECOLOGICAL EFFECTS

The deliberate or unintentional release of genetically modified organisms may directly or indirectly lead to changes in the natural environment. The environmental effects may be broadly divided into three types (after Williamson *et al.* 1990): (1) effects caused by the genetically modified organism itself, (2) effects resulting from dissemination of genes from the genetically modified organism to other organisms in the environment, (3) changed practice in the use of an organism because of the genetical modification (for example, a different way of using herbicides after changing to herbicide-resistant plants).

The predicted biological effect of a deliberate release is usually specific with regard to species, environment and period of time. Evaluations of environmental impacts must, however, also include other species and environments, because both the genetically modified organisms and their genes can spread to other environments as well as within the environment in which they are being used. Assessments of environmental impacts must, moreover, have a long-term perspective, because the release concerns living and, frequently, reproducing organisms (TeknologiNævnet, 1990). Providing documentation on the environmental effects of a deliberate release will therefore always be a major task, but it will be almost impossible to predict them.

This chapter will discuss some real or potential dangers in the deliberate release of genetically modified organisms. Since experience is still too limited to justify the drawing of conclusions on such effects, examples of environmental effects from the deliberate release of other, non-modified, organisms will also be given.

2.1 Potential risks of introducing genetically modified organisms

Genetically modified viruses

The general risks of introducing genetically modified viruses include the possibility for recombination with related, naturally occurring, viruses, dispersal by insects, insertion of virus DNA into cell chromosomes, and establishment of long-lasting infections within one or more populations in the ecosystem.

When assessing the environmental effects of viruses that have been deliberately released or have escaped, it is important to take into consideration that a virus particle that infects a receptive host cell can give rise to millions of identical virus particles in the matter of a few hours. A single unintentional mutation or a single unforeseen recombination can therefore lead to completely unexpected consequences.

Some viruses are apparently only capable of infecting pecific types of cell in one or a few closely related host species. Other viruses have a broad host range at both species and cell levels. At the species level, there may be a significant variation in receptiveness between individuals, from totally unreceptive to completely receptive. The variation may be related to sex, age, genetical mosaic, the phase in the cell cycle, or to which other viruses and micro-organisms the individual is currently acting as host. Which host-cell genes are expressed at the moment of infection may also determine the entire course of a virus-host confrontation. The receptiveness of a species, an individual or a cell to a given virus is consequently most decidedly a relative concept.

Small genetical changes right down to point-mutation level, may, in a given virus, lead to important changes in host specificity and other properties. This applies to both protein coding and non-coding viral gene sequences.

In addition to the phenotypical traits that have been consciously changed, genetically modified viruses may therefore have been given properties that can have great ecological significance and that can only be revealed through very comprehensive and well planned experiments. In many cases, the complexity of the ecosystems will preclude adequate investigation.

In the case of viruses now under assessment for deliberate release, little knowledge generally exists about the occurrence of natural relatives in the ecosystems. It is therefore impossible to predict which recombinations may occur. However, in the case of the Poxviridae family, to which the vaccinia virus belongs, recombinations producing viable progenies have been demonstrated between several, sometimes distantly related, species (Kaplan, 1989). Recombinations between non-related viruses may also be more common than hitherto recognised; for example, the transfer of genetical material between human adeno-associated virus type 2 (Parvoviridae) and human herpes virus type 6 has recently been demonstrated (Thomson *et al.*, 1991).

During trials with a baculovirus there is a risk of the virus attacking other insects than those intended. This can probably take place even if only a single gene of the virus is changed (Royal Commission, 1989). When a genetically modified baculovirus underwent testing in Great Britain, a "destroyed" genetically modified virus was used that was still pathogenic for the insect pest, but that could not survive in the environment following the infection (Bishop, 1988). This was thought to reduce the probability of other organisms than the insect pest being attacked, but it does not eliminate the risk.

Norwegian research workers have recently reported much higher concentrations of viruses in aquatic environments than hitherto assumed (Bergh *et al.*, 1989). They stress that the high concentrations make it likely that genes from any deliberately released, genetically modified micro-organisms can be spread to local, natural bacteria (Bergh *et al.*, 1989).

Genetically modified micro-organisms

Following lengthy debate, the first deliberate release of genetically modified micro-organisms was undertaken in the USA in 1987. In this experiment, strawberry plants were sprayed with "ice-minus" variants of the bacterium *Pseudomonas syringae* with the objective of reducing frost damage (see above). Some workers claimed that large-scale use of "ice-minus" bacteria could lead to climatic change. The United States Congress Office of Technology Assessment examined this assertion and concluded that the risk of climatic change was negligible, both globally and locally (OTA, 1988). The deliberately released "ice-minus" bacteria depend on plants for their survival. The trial was therefore made safe by laying fallow an area of land around the experimental plot (using bare earth) and destroying all the plants at the termination of the trial. While the experiments were taking place, no genetically modified bacteria were found beyond the zone of bare earth, or on other plants than the experimental ones (Gaertner & Kim, 1988).

The risk of changes in important biogeochemical cycles was assessed in connection with trials using genetically modified, nitrogen-fixing bacteria. OTA (1988) concluded that the risk of detrimental environmental impact was remote, and insufficient to prevent the trials commencing. A form of background experience existed already in that non-engineered *Rhizobium* bacteria had been deliberately released in several parts of the world for over 100 years, to enhance nitrogen uptake in nitrogen-fixing plants, without any known, dramatic environmental effects (Royal Commission, 1989).

Experiments involving the introduction of toxin genes (e.g. Bt toxins) into micro-organisms or cultivated plants to combat insect pests must be assessed from several angles: 1) the genetically modified organism may be poisonous to other creatures than the pest; in particular, the effect on insects which are important for insect-pollinated plants should be studied, 2) the toxin may be poisonous to people, and 3) with large-scale use, the insects may develop a resistance to the toxin (Royal Commission, 1989).

Genetically modified plants

Several reports have put forward the evolution of virulent weeds as the potentially greatest threat when deliberately releasing genetically modified plants. This may take place either by such plants spreading unaided from their cultivated position to become established as weeds, or by transfer of genes from the cultivated plants to closely related species by hybridisation. The risk will be greatest if the plants are given qualities that can provide them with enhanced ability to compete or adapt (e.g. tolerance to salt or drought, resistance to herbicides, etc.).

The danger of new virulent weeds being evolved has also been a general risk in conventional plant breeding through the introduction and uncontrolled dissemination of new cultivated plants. Many domesticated species introduced into North America and Australia from Europe during the colonisation period have become established as weeds. Pimentel (1986) reports that 73 % of the 80 worst arable-land weeds in the USA have been introduced, the corresponding figure for weeds in pastures and natural grassland being 41 %.

Many different properties cause specific plant species to become virulent weeds (Baker, 1965; see Rognli 1991). It is therefore impossible to point to particular traits that characterise all weeds.

In many cases, only a few genes distinguish cultivated plants from weeds, and weeds have frequently evolved from cultivated plants (Keeler, 1985). With existing knowledge, it is impossible to claim that there is a greater probability of genetically modified plants developing into weeds than plants introduced or bred in a traditional manner. The probability for mutations that can give rise to weeds is greatest if the weed characteristics: 1) are controlled by few genes, 2) require loss of genetical control (new mutation), rather than mutation back to a specific gene, or 3) are dominant characters. The weed characteristics chiefly seem to be recessive mutations, and mutations in single genes are known which give several of the characters that typify weeds (Keeler, 1985). So far, only single genes have been transferred to plants by means of genetical engineering. It is uncertain whether the introduction of single genes can lead to cultivated plants evolving into weeds. Mutation and recombination in such inserted genes, with resultant natural selection, therefore seems to be one of the few conceivable ways of evolving a virulent weed from a genetically modified plant.

Based on a comparison of the characters found in three groups of plants - virulent weed species, a random selection of species and the most important cultivated species - Keeler (1989) claimed it to be extremely unlikely that genetically modified variants of relevant cultivated species can evolve into weeds. It is, however, emphasised that a great deal is unknown concerning the evolution of weeds. Well-known cultivated species that have little dispersal ability and lack wild populations that are weeds will be unlikely to develop into weeds themselves. Species with high dispersal ability and that already include weed populations, on the other hand, constitute a much greater threat.

Generally speaking, the risk of gene dispersal from genetically modified plants is greatest in wind-pollinated species that are able to cross with wild populations of the same or closely related species, the hybrids giving viable progenies. Typical species of this kind are grasses and woodland trees. The risk of hybridisation is extremely small in species that are strictly self-pollinating, such as the most important cereals in Norway. In general, it may be said that the risk of gene dispersal through hybridisation is greatest in species which have their genecentre within the cultivated area concerned (e.g. grass species in the case of Norway).

Horizontal transfer of genes from plants to other organisms, e.g. bacteria and viruses, constitutes a potential threat for dispersal of genes from genetically modified plants. Such gene transfer has so far not been proved, but it has nonetheless been shown that gene sequences are to be found in plants that are very similar to gene sequences in micro-organisms and animals. The structure of the genetic material in mitochondria and chloroplasts in plants suggests that early in their evolution eucaryotic cells have assimilated procaryotic cells (blue-green algae and bacteria). Similarly, the structure of leghaemoglobin in leguminous plants, which plays an important role in nitrogen fixation, is so similar to haemoglobin in animals that the gene probably has a common origin far back in evolution. A gene sequence has been found in species of the tobacco genus, *Nicotiana,* that is also found in *Agrobacterium rhizogenes* and that probably at some time was transferred from *Agrobacterium* to the tobacco species by natural transformation (Furner *et al.,* 1986). Little is known about the scale and speed of such processes in the natural environment.

Most of the cultivated plants in the world originate from tropical and sub-tropical regions, where they are today often cultivated side by side with their wild relatives (Harlan, 1975). Hence, even though many genetically modified cultivated plants can be grown in Norwegian latitudes with minimal danger for transfer of genes to wild relatives, there is a risk of such transfer taking place in the tropics and sub-tropics (Ellstrand, 1988; Tiedje *et al,*. 1989).

The cultivated plants that are most likely to be affected by gene transfer (e.g. potato, tomato, rape and cereals) have few or no wild relatives in Norway (Elven *et al.*, 1991). Even though the risk of genes being transferred is therefore minimal, the probability of dispersal taking place will increase when the number of deliberate releases becomes large, and when the releases take place on a large scale. If the species is able to transfer its genes to surrounding vegetation it must be expected that gene dispersal will eventually take place. This does not mean that the spreading of genes will necessarily have ecological impacts; that must be evaluated on a case-by-case basis.

When assessing the ability of a genetically modified plant to survive and compete, it is useful to base the evaluation on a schematic life-cycle of a plant, as shown in Fig. 1. In the figure, the stages in the life-cycle that are important for dispersal are marked with an asterisk. For most cultivated plants, we have too little knowledge about how long seeds and vegetative reproductive organs survive in the soil. The measures needing to be introduced to hinder dispersal of reproductive organs depend on the type of plant. It may be necessary to monitor the experimental plot over several seasons following a deliberate release, to prevent the establishment of surviving seeds or vegetative organs.

Genetically modified animals

It is possible to genetically modify invertebrates for pest control purposes (Gaugler, 1987; Yoder *et al.*, 1987). In the Nordic countries, non-modified nematodes (roundworms), mites and insects are used to combat insect pests, chiefly in greenhouses (Mehl, 1991). They are bred in a few large factories for distribution and deliberate release. Abroad, nematodes, and insects such as ladybirds and green lacewings, are produced for release outdoors. The same insect species are useful as aphid predators on cultivated plants in Norway, too, and nematodes are used as predators on insect larvae living in the soil in greenhouses and outdoors.

When animals are being genetically engineered for biological control purposes, it is desirable to increase their efficiency in controlling pests and improve their adaptation to the climate and environment in which they are to be released. Examples of traits whose modification is desired include enhanced reproductive ability, shorter generation span, greater mobility, more killed hosts per predator, broadened host and prey ranges, and lower temperature limit for reproduction and activity.

Genetically modified insects, nematodes and other invertebrates can be expected to have the same environmental impacts as when the same organisms are used (without modification) in classical biological control operations. It can, moreover, be assumed that broadening the host and prey ranges, for instance, will pave the way for substantial damage, since it will be difficult to predict which insect species they will attack in the natural environment.

Hope has been expressed that genetical engineering can be employed to control, for instance, the malaria-carrying mosquito more effectively than today (Eggleston, 1991). Concern has also been expressed that some of the negative side effects of biological control (uncontrolled dispersal, loss of other species than the pest, etc.) will be unavoidable if the risk assessments prior to deliberate release are not sufficiently adequate (Howarth, 1991).

A problem that arises when the environmental impacts of genetically modified invertebrates are being evaluated is that, for many of these animal groups, very little is known about which species occur in Norway. It is therefore difficult to estimate the prospects for establishment and dispersal of genetically modified invertebrates in the natural environment in Norway, and also to determine whether they can transfer their genes to closely-related populations or species.

The domestic animals that are relevant for genetical engineering are unlikely to represent any environmental threat. Sheep that are genetically modified to produce a blood-coagulating human protein will probably not have other environmental impacts than non-modified sheep, and the sheep as a species has so far not formed a wild stock in Norway.

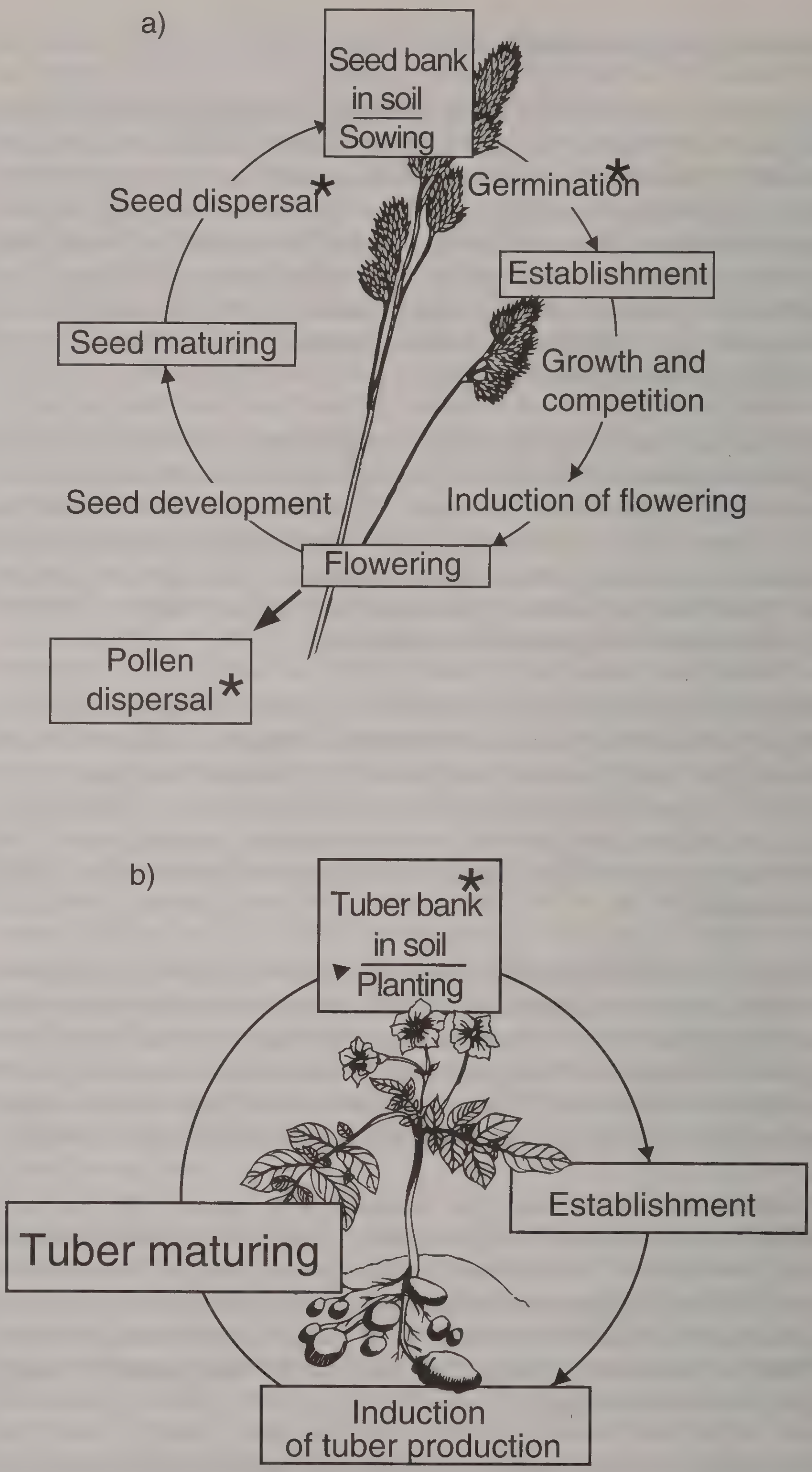


Figure 1. *Schematic life-cycles in plants reproducing by seed pollination (a) and vegetative organs (b). Stages where a risk exists for dispersal of the plant itself, or its genes, are marked with an *.*

Fish that are genetically modified for use in aquaculture may affect the environment in a variety of ways if they escape on a large scale. Among these impacts are competition with, and disease transmittance and transfer of genes to, populations of the same or closely-related fish species (see section 2.2). Since all except one (rainbow trout) of the species that are relevant for Norwegian aquaculture have wild populations in Norway, the impact of the dispersal of genes from farmed to wild populations must be evaluated. Sterile fish can now be produced on a large scale using simple, cheap technology. This can prevent transfer of genes between escaped farmed fish and wild fish, but will be unable to prevent competition with, and transmittance of disease to, wild fish. The committee therefore emphasises the importance of solving the present problem of fish escaping from marine aquacultural facilities before considering basing production on genetically modified fish.

2.2 Comparison with introduced species

Nothing is yet known about how a GMO will behave over time in the natural environment. In reports prepared internationally about the deliberate release and potential environmental effects of GMO's, considerable emphasis has therefore been placed on experience gained following introductions of exotic species into new environments.

Introduction of species

Introduced species can transform entire ecosystems. The character of the Australian flora and fauna is completely different now than before Europeans brought plants and animals with them to the Australian continent (Groves & Burdon, 1986). The proportion of introduced species that succeed in establishing themselves is not high. Williamson & Brown (1986) have estimated that 10 % of the species introduced into Great Britain have become established, and 1 % of the introduced species (i.e. one-tenth of those that have become established) have attained pest status. Studies on introductions of mammals and birds to areas where they were not previously found suggest that every fifth deliberate release which leads to establishment has substantial ecological impact (Ebenhard, 1988; Simberloff, 1991).

The introduced species may have significant impacts on local species through being vectors for parasites and diseases, competitors, and herbivores or carnivores. In some cases, the introduced species are able to hybridise with local, closely-related species. Based on experience gained from deliberately releasing species, attempts have been made to list some general indications of risk, including the following. Uncontrolled dispersal of the introduced organism more often takes place in the case of organisms with high reproductive rates, asexual reproduction or self-fertilisation, long life spans, small fluctuations in numbers, and good opportunities for local dispersal. Dispersal, moreover, occurs more readily when the deliberate release has taken place into an environment that contains little biological competition (i.e. one with few species, or having a young or "disturbed" ecosystem, for example because of human activity).

It is not known which species have reached Norway in a natural manner or been introduced by people without succeeding in becoming established. However, examples are known from many groups of organisms of introduced species that have become established and have spread in Norway, influencing the existing plant and animal communities to greater or lesser extents.

Viruses, bacteria, fungi and parasites which cause diseases in fish may have been introduced into Norway. Examples include the bacterium *Aeromonas salmonicida* which causes furunculosis in salmonoids, the monogenean *Gyrodactylus salaris* which also attacks salmon, and the fungus *Aphanomyces astaci* which is the cause of crayfish pest.

There are also examples among cultivated plants of introduced species that have established and dispersed themselves beyond the areas in which they were, or are, being cultivated. One is Reed Sweet-grass *(Glyceria maxima)*, which was probably introduced into Norway as a fodder grass in the 18-19th centuries and which is now an important component of swamps in the lowlands of southern Norway (Elven *et al.*, 1991).

Pineapple Weed (*Matricaria matricarioides*) is one of the worst weeds in Norway today. It is an exotic species introduced to the Botanical Gardens at Tøyen in Oslo in 1862 from where it has spread over the whole country (H. Fykse, the Norwegian Plant Protection Institute, pers. comm.).

Canadian Waterweed *(Elodea canadensis)* was introduced from North America to Europe in 1836, reaching Østensjøvannet in Oslo, in 1925. It fills the littoral zone of lakes in which it becomes established, such as Jarenvannet and Steinsfjorden, and can alter the phosphorous exchange so that the "internal fertilisation" of the lake increases resulting in eutrophication (Rørslett & Berge, 1983). Canadian Waterweed spreads to new watercourses with the (unintentional) help of man, through the moving of boats, fishing tackle, etc., and is still being registered in new watercourses in southeastern Norway (Bjørn Rørslett, NIVA, pers. comm.).

The Crucian Carp *(Carassius carassius)* is a species of fish that has become established in many places where it has been deliberately released, but it is not known whether it has spread of its own accord. Among birds, the Canada Goose *(Branta canadensis)* has become established and spread following introduction. The Mink *(Mustela vison)* has become a prominent feature of the mammal fauna, and has considerable impact on other communities. Introduced species have also succeeded in becoming established in the most arctic area of Norway, on Svalbard. For instance, the previous keeping of cattle was probably responsible for introducing parasites that have become established in the Svalbard Reindeer (Bye *et al.*, 1987); the Sibling (or Russian) Vole *(Microtus epiroticus)* has also established several colonies on Svalbard following its introduction, probably by Dutch whaling vessels as early as the 17th century, and may be the most abundant terrestrial mammal on Svalbard today (Yoccoz *et al.*, 1990).

Deliberate releases of alien stocks of the same species

Deliberate releases of an organism into localities where locally adapted stocks of the same species already exist can lead to spread of disease and loss of the local stock, changes in the genetical structure of the species and loss of local adaptations (Templeton, 1986; Hindar *et al.*, 1991). This particularly applies to species that are highly structured in local and, as regards reproduction, more or less isolated stocks, such as salmonoids (Ryman & Utter, 1987).

Experience gained from the deliberate release of salmonoids suggests that the genetical and ecological effects are unpredictable and variable, from no demonstrable impact to substantial hybridisation, spreading of disease, and competition. There are many examples of deliberately released fish from an alien stock, and hybrids of released fish and local ones, performing less successfully in the natural environment than the locally-adapted stocks, thereby reducing the productivity of those stocks and their ability to adapt in the longer term (Hindar *et al.*, 1991).

3. ASSESSING THE RISK OF UNDESIRABLE ENVIRONMENTAL IMPACT

There are both theoretical and empirical grounds for claiming that it is not yet possible to predict the genetical and ecological consequences of the deliberate release of genetically modified organisms into nature. Theoretical, because the information required to enable good predictions to be made is in practice unknown (genetical variation in the species and the forces that influence it, possible interactions between species and the environment); empirical, because experience from introductions of non-modified organisms has revealed many environmental effects that were neither intended nor foreseen (Drake *et al.*, 1989).

General guidelines have been proposed for assessing the environmental impacts of a genetically modified organism. It is, however, clear that detailed knowledge about the genetical modification, the host organism, the environment in which it is deliberately released and other aspects related to the release (including the number of individuals) is necessary to enable biologically justifiable risk assessments to be made. These assessments must also include effects on other environments to which the genetically modified organism is able to spread, and they must have a long-term perspective. Pilot trials in contained environments form an absolutely essential part of risk assessments prior to introduction of genetically modified organisms into the natural environment.

3.1 Two principles when assessing risks

American researchers have proposed applying the following principle for evaluating the risks when genetically modified organisms are to be deliberately released:

> ***"Genetically engineered organisms should be evaluated and regulated according to their biological properties (phenotypes), rather than according to the genetic techniques used to produce them" (Tiedje et al. 1989).***

This means that, as regards environmental threat, there may be no difference between deliberately releasing genetically modified organisms and other organisms. Other workers have pointed out that the technique used in the genetical engineering must also be assessed. For instance, it is at present extremely difficult to control where in the genome of animals and plants an inserted gene will ultimately end up. The position may have consequences for the stability of the genome, first and foremost with respect to regulation of gene expression and the activation of genes that are not usually expressed (Kjelleberg & Fagerström, 1990).

In this context, the difference between coding and regulatory sequences of DNA is important. A coding sequence produces a specific genetic product (e.g. a protein), but whether the gene becomes silent or active, and the degree of its activity, is determined by the position of the gene in relation to regulatory sequences. Given types of regulatory sequences can influence the activity of many coded genes, and over long distances on the chromosome. If a regulatory sequence also affects genes which code for genetically regulating molecules (e.g. transcription factors, hormones, growth factors, proto-oncogenes, etc.), the activity of genes on other chromosomes in the same cell, and the genetic activity of other cells may also be affected. Regulatory sequences are under the constant influence of environmental factors in the cells and the surroundings of the cells, such as temperature, pH, chemicals, heavy metals, products of other cells and micro-organisms.

When genetically modified organisms and viruses are being constructed, not only coding sequences but also regulatory sequences are transferred, the latter to ensure the proper expression of the inserted gene. To be able to assess the expression of both the inserted gene and the genes of the organism itself under varying environmental conditions, the location of the introduced regulatory sequences in relation to the original genes of the host must be mapped. At present, this is only possible, in practice, in the case of viruses and well-known micro-organisms. Deliberately released viruses contain regulatory sequences that can be integrated into the DNA of the host cells and influence genes in the manner described earlier. One theory currently gaining support proposes that changes in regulatory sequences have been, and are, more important for evolution than changes in coding sequences (Paigen, 1986).

Methods are being developed to determine beforehand where foreign DNA must be inserted into cell DNA (gene targeting). By employing such methods in the construction of GMO's and viruses some of the problems associated with insertion of regulatory DNA sequences can be controlled. But there are still few examples of the successful employment of gene targeting (Singer & Berg, 1991).

It has been suggested that genetically modified individuals with additional (inserted) DNA carry a physiological burden which makes them less well adapted than their non-modified relatives in environments that are not changed to favour them (Lensky & Nguyen, 1988). This is, at best, an imprecise generalisation; there is no law in nature that states that a great deal of "unnecessary" DNA reduces the ability of the organism to adapt, and some experiments show that genetically modified organisms can have a higher reproductive and survival ability than their non-modified relatives (Hartl *et al.*, 1983; Royal Commission, 1989). In addition, it is important to bear in mind that also genetically modified organisms are exposed to natural selection and will be able to evolve better adaptations to the environment in the long run.

There will always be an element of biological gambling involved in the deliberate release of genetically modified and other (non-modified) organisms. The level of knowledge in the fields of genetics and ecology is inadequate to permit the making of precise, *a priori* risk assessments for deliberate releases (Simonsen & Levin, 1988; Drake *et al.*, 1989; Hindar *et al.*, 1991). General guidelines on how to assess environmental effects can be provided, but sufficient knowledge of the ecological interactions and the genetical basis for these is unlikely to be obtained to support the provision of precise predictions. This is because we know neither the genes that are important for adaptations to the environment nor the selective forces acting on these genes. Many other items of information that are important for making precise genetical and ecological predictions (including population size, exchange of individuals and genes between natural populations, and the food choice, natural enemies and evolutionary history of the species) are also unknown quantities in nature - even in well-known groups of organisms.

The necessary ecological and genetical knowledge to make precise risk assessments is unlikely to be obtained within the foreseeable future. Hence, the best we can do is to base our opinions on experience and then give *a posteriori* risk assessments that are as precise as possible.

An important principle is therefore: *"environmental effects of deliberate releases of genetically modified organisms must be reviewed on a case-by-case basis through a stepwise procedure that includes contained pilot experiments on a variety of scales"* (OECD, 1986).

There is broad international agreement about this principle (Royal Commission, 1989; Tiedje *et al.*, 1989). Some maintain, however, that it will be difficult to carry out such a system in practice when the number of applications for deliberate release become large (Stearns *et al.*, 1988), and therefore wish to classify such releases in different risk categories. As has been shown above, scientific evidence exists that this is not possible. It has in fact been suggested that the worst ecological problems may come from releases that are looked upon as safe, because these are not carried out using the same safety measures as the assumed risky releases (Crawley, 1988).

The committee wishes to emphasise that even a risk assessment based on the principles of "case-by-case" and "step-for-step" has limitations such as (1) short-term experience must be used to assess long-term impacts, (2) we are only able to test the possible problems we are aware of, and (3) inability to prove any impact may be caused by problems with the method or be the result of the investigation not covering all the relevant effects (Simonsen & Levin, 1988). Moreover, in many cases of deliberate releases it has been shown that the result very largely depended upon how many individuals were released (Drake *et al.*, 1989; Griffith *et al.*, 1989). The results of small-scale trials need not therefore be directly transferable to large-scale experiments.

Against that background, it is important that deliberate releases of GMO's are not undertaken without the benefit being well documented, as is emphasised in White Papers nos. 8 and 36 (1990-91) on biotechnology.

3.2 Use of mathematical models in risk assessments

Mathematical models for invasions can be used to describe the general features of invasions, explain past invasions, and predict something about the course of the invasion when one occurs. We lack, however, the necessary ecological knowledge to enable predictions to be made in individual cases (Williamson, 1989). There are also grounds for emphasising that even if our understanding of ecology were sufficiently good to enable us to construct "accurate" mathematical models with correct parameter values, the variability of nature would always limit the possibilities of predicting what would happen in a single deliberate release. But the use of models can help us to understand how various environmental impacts can arise, particularly when we are assessing the risk of a specific environmental impact taking place. An overview of some mathematical models that are used is given by Hindar & Bakke (1991), with examples from deliberate releases of GMO's.

The phases of an invasion for which it is natural to study models in connection with the deliberate release of genetically modified organisms are 1) establishment and 2) dispersal.

Establishment (colonisation)

For a species to be able to invade an ecosystem, it must first be introduced into the ecosystem and then establish itself. In field trials involving deliberate releases, the introduction will be monitored by those carrying out the trial, and the establishment (colonisation) will therefore be the first stage in the invasion for which it is interesting to study models.

One type of factor that can be included in models for establishment concerns the establishing population, e.g. its size and growth rate. Models show that in the establishment stage, small populations are more vulnerable than larger ones (Allee *et al.*, 1949). Disagreement, however, exists about the importance of the size and growth rate of a population for its ability to establish.

Another type of factor concerns the habitat in which the establishment is to take place. Some models predict that the habitat must be larger than a certain minimum size if a given species is to be able to invade it. Simulation models exist for studying establishment in habitats with an existing food chain.

The theory for establishment is not general, but is developed for particular types of populations and interactions between populations. It has, moreover, been little tested (Roughgarden, 1986). The best developed establishment models are those for epidemics. Subject to certain assumptions, they can provide answers to such questions as how large a population must be for an epidemic to gain a foothold.

Dispersal

An invading organism that has become established will usually spread. Dispersal depends upon survival, reproduction and movement. An important quantity that enters into dispersal is speed. An organism that spreads slowly can probably be controlled better than one that spreads quickly. Models for dispersal are better developed and tested than models for establishment. However, models for establishment seem to be of greater value for estimating the invasion ability of an organism than models for dispersal (Williamson, 1989).

The types of dispersal models that have attracted most attention are diffusion models. Dispersal is described in these models by an equation in which diffusion and population dynamics are included. In the simplest case, diffusion can be thought of as having occurred by each individual moving independently of the other in small steps, the direction of each step being random and the population growing exponentially. This simplest conceivable equation has, for instance, been used to describe the spread of the Muskrat (*Ondatra zimbethica*) in Europe (Skellam, 1951).

In the case of theoretical models applied to the deliberate release of genetically modified organisms, it is also important to introduce the concept of survival. Theoretically, genes, micro-organisms and viruses can survive long periods in concentrations that cannot be proved by biological, genetical or immunological tests. When conditions in the ecosystem are changed, establishment and dispersal can take place. It may then be too late to intervene (Goksøyr & Sørheim, 1991).

3.3 Criteria for risk assessments

A number of general criteria have been set up to assess the risks involved when genetically modified organisms are released. These can be arranged under 1) the genetical modification, 2) the host organism, 3) the genetically modified organism compared with the host organism, and 4) the environment into which the deliberate release is made (see Table 4). Each of the criteria for risk assessment mentioned in Table 4 should be included in a survey of the possible risks entailed in deliberately releasing a genetically modified organism. The grading from "safe" to "risky", however, varies according to the different scales used for the various criteria, which cannot therefore be directly compared. Moreover, our background knowledge for many of the criteria, particularly concerning the characteristics of the organism and the environment into which it is being released, is so poor that the list of criteria can easily give a false sense of security regarding our capability of ranking genetically modified organisms according to the degree to which they are a danger to the environment (Kjelleberg & Fagerström, 1990). For instance, Williamson *et al.* (1990) point out that even a detailed list of the characteristics that differentiate weeds from other plants in Great Britain only classifies 6 of 7 species in the correct group.

It has been argued that since gene technology is often no more than a copy of the natural gene exchange of micro-organisms[1)], new and unknown environmental dangers will not arise when genetically modified organisms are deliberately released (see, for example, Davies, 1988). This is an over-simplification that fails to take into account that the scope and consequences of the gene exchange vary with the place, the time and the number of micro-organisms. Until there is adequate information on the extent of gene exchange between micro-organisms under natural conditions, only laboratory experience is available for risk assessments. The few field trials carried out suggest that laboratory experience gives at best an extremely over-simplified view of the threats to the environment when deliberate releasing is being undertaken (Miller, 1988).

To improve safety, the following is some of the information that should be known about GMO's and viruses before they are deliberately released into the natural environment:

- *detailed mapping of the location of the inserted gene in relation to cellular genes,*
- *the behaviour of the regulatory sequence under varying conditions, and the consequent opportunities for influencing the expression of the inserted gene and the cellular genes,*
- *whether the inserted gene can, under given conditions, lead to local increase (amplification) of DNA,*
- *whether the inserted gene can lead to movement of DNA from one chromosome to another (translocation),*
- *whether the inserted gene can come into contact with reverse transcriptase activities, with the consequent opportunity of being inserted at new sites in the cell DNA and also of being dispersed by viruses and micro-organisms,*
- *whether dispersal of genetic material can take place through several steps before it becomes expressed (e.g. from bacterium to mycoplasma to eucaryotic cell),*
- *whether a genetically modified virus can be integrated into cell DNA, or recombine with a naturally occurring virus which can integrate,*
- *whether viruses or micro-organisms have had their host range broadened to include new species in the ecosystem, or been made capable of using new means of dispersal (e.g. insects).*

---

1) Several of the techniques used in gene technology occur naturally in micro-organisms (Miller, 1988). Gene transfer between bacteria can take place by (1) conjugation, i.e. that the bacteria exchange plasmids through direct cellular contact (plasmids are small DNA molecules outside the chromosome), (2) transduction, i.e. genes are transferred via bacteriophages (bacteria viruses), and (3) transformation, i.e. the bacteria take up naked DNA directly from the environment. DNA can, moreover, be re-located within the cell (for example, it can "jump" between plasmids and the chromosome) as a result of the action of transposons. These processes are well known from bacteria in laboratory experiments, and must have contributed to genes which provide antibiotic resistance being dispersed to a large number of different bacteria. However, extremely little knowledge is available about the extent to which this process occurs in nature (Miller, 1988; Kjelleberg & Fagerström, 1990).

Many more desirable items of information might be mentioned, and many of these desires can clearly never be fulfilled because the complexity of ecosystems cannot be imitated in experimental models.

The committee is of the opinion that all genetically modified viruses should be mapped in detail, i.e. by sequencing the entire genome. In the case of well-known micro-organisms, a description that is as detailed as possible should also be available. For higher plants and animals it is not feasible in practice to demand sequencing of the entire genome. Nor is it certain that such sequencing, if available, would improve the risk analysis. The way development is proceeding, it will probably soon be possible to demand documentation of where the inserted genes are located in the genome, and the sequences of the neighbouring genes.

Table 4. *Criteria for risk assessments, based on an overview prepared by the Ecological Society of America (adapted from Tiedje et al., 1989).*

Genetic modification	***Host organism or parent organism***	***Modified organism compared with host organism***	***Release environment***
The more fully characterised, the safer.	The more domesticated, the safer.	Lower adaptability safer than higher adaptability.	Safer if: - there is no selection pressure for the new trait,
The more genetically stable, the safer.	Controllable species safer than non-controllable ones.	Lower pathogenicity safer than higher pathogenicity.	- if there are no relatives of the modified organism in the vicinity,
Removal of genes is safer than introduction of genes.	A local species is safer than an alien one.	Unchanged host range is safer than changed or broadened host range.	- no opportunities for dispersal are provided,
Non-functional genes are safer than functional ones.	Free-living species are safer than pathogens, parasites or symbionts.	Reduced environmental tolerance is safer than enhanced environmental tolerance.	- the organism is not a key species in the environment,
Re-introduction into the same host is safer than gene transfer to a new host.	Species lacking pest status are safer than species with pest status.	Reduced resistance to diseases, parasites and predators is safer than enhanced resistance to them.	- the organism has no alternative hosts,
Introduction without a vector is safer than introduction with a vector.	Species without special survival stages are safer than species with special survival stages.	Enhanced opportunity for control is safer than reduced opportunity.	- the organism has a small potential area in which it can exist,
A vector from the same species is safer than one from non-related and/or pathogen species.	Species with narrow ecological tolerance and geographical range are safer than species with broad tolerance and geographical range.	Stable gene expression is safer than environmentally dependent gene expression.	- the environment can be realistically simulated prior to deliberate release,
A non-functional vector DNA/RNA in a GMO is safer than a functional vector DNA/RNA in a GMO.	Species with little natural gene dispersal are safer than species with high natural gene dispersal.	Similarities to safe modified organisms are better than no similarities to them.	- there is documentation that the environment can be monitored and controlled.

3.4 Special features of environments for deliberate release in Norway

Agriculture, aquaculture and forestry in Norway operate under very special natural conditions (e.g. day-length and temperature) that differ greatly from those in countries leading the way in the development of gene technology. Nowhere else in the world is economic exploitation of biological production carried on as far north as in Norway. This means that genetically modified organisms adapted to these primary industries abroad may either show entirely different environmental impacts in Norway, or completely lack the ability to adapt and survive here.

Large areas of Norway have arctic and sub-arctic ecosystems. More than 90 % of cultivated ground in such areas consists of grass. Species diversity in the natural ecosystems is low, strong selection pressures operate and the ecosystems are considered to be vulnerable. As mentioned earlier, the probability of introduced species establishing themselves is high in ecosystems that have few species and/or are exposed to anthropogenic disturbance. The question may be asked to what extent this holds for ecosystems in Norway. As regards plants, it is obvious that under extreme climatic conditions the distribution of the populations is to a greater degree determined by a few physical factors in the environment, for example, temperature, than by biological competition, as is the case in optimal environments. The few species that have succeeded in adapting themselves to such extreme areas have been exposed to strong natural selection and have special characteristics (e.g. they are perennials, are polyploid, or can reproduce vegetatively). The genetical control on adaptation to extreme climatic conditions is highly complex. Against such a background, it may be argued that there will be little danger of introgression of genes from GMO's, or that GMO's which become wild following deliberate release into such environments will be able to influence the plant communities. But it must be emphasised that the effects will depend upon the rate of introgression.

Norway also has many areas where natural vegetation occurs close to cultivated ground. This ground is located in an extremely dispersed fashion and does not form large continuous areas of agricultural landscape, as in the major agricultural countries. Hence, the risk of spreading genes from GMO's to plants of the same species or to close relatives found in the natural vegetation will be considerable.

Forestry and in part also cultivation of meadows and grazing land take place under almost natural conditions with few opportunities for controlling the environmental conditions. For example, the control of insect pests in forests using GMO's may substantially affect other insects, particularly if GMO's with a broadened host range are used.

Substantial economic interests are attached to the marine environment in Norway, both as regards wild and farmed fish. Many examples of environmental catastrophies following deliberate releases into aquatic environments suggest that these environments are especially vulnerable (Miller, 1989; Baltz, 1991).

4. REGULATING MODERN BIOTECHNOLOGY

Three main models are used when the deliberate release and use of genetically modified organisms are being regulated. The first has no legislation controlling such release of genetically modified organisms, but has guidelines which the authorities recommend be used (e.g. Australia). The second employs a modification of existing laws and regulations to cover regulation of gene technology, and new regulations are also drawn up to fit into existing legislation (e.g. the USA). In the third model, new legislation is created specifically for gene technology, regulating that collectively in relation to the environment and safety (e.g. Denmark).

In the following sections, two of these models, using the USA and Denmark as examples, are described, along with the strategies in the EC and OECD. Finally, portions of the proposal put forward by the authorities for a Norwegian regulation are evaluated.

4.1 International regulation

Regulation in the USA

The USA has no uniform legislation regulating biotechnology, and responsibility for its regulation is largely divided within the administration according to the type of product to be regulated. No single public institution has management authority over all the diverse product areas embraced by biotechnology. Responsibility for managing biotechnological activity is therefore divided between several public institutions.

All laboratory or contained research with genetically modified organisms that receives financial support from the EPA (Environmental Protection Agency), USDA (United States Department of Agriculture), NIH (National Institute of Health) or other public institutions must comply with the NIH guidelines unless regulations are in place for agency oversight. All testing of organisms in the environment, including GMO's, is primarily under the jurisdiction of EPA or USDA regardless of whether it is funded by public institutions or private industry. The exception to this is EPA oversight under the Toxic Substances Control Act which, under current policy, asks for voluntary reporting of research involving environmental uses of micro-organisms.

The US policy is described in the "Coordinated Framework for Regulation of Biotechnology" published in 1986 by the federal government (Food and Drug Administration, EPS, USDA, Occupational Safety and Health Administration, and NIH). This policy places emphasis on a unified public policy and coordination of procedures for both contained uses and deliberate release of GMO's into the natural environment. The laws and regulations are, in general, based on a product-by-product basis. In general, the policy of the public institutions is to follow a "case-by-case" handling of applications for such release. But the USDA and EPA can exempt certain categories of organisms from regulation (United States General Accounting Office, 1988).

Regulation in Denmark

In 1986, the Environment and Gene Technology Act was put on to the statute book in Denmark. This legislation gave the Secretary of State for the Environment the responsibility and ultimate authority concerning matters within the sphere of gene technology. The control function here was subsequently delegated and is now the province of the National Agency for Environmental Protection which is a directorate under the Ministry of the Environment. Because of the needs of the Danes themselves, and with background in EC directives, a revised draft of the Act was put forward in autumn 1990. This was adopted by the Danish parliament in June 1991.

Some important points in the Danish Act are:

- Permission to deliberately release GMO's will be considered on a case-by-case basis.
- Research in which genetically modified organisms are produced or used must only take place in laboratories or within laboratory areas that are approved for such use.
- Classification is required for contained use. Based on the properties of the organism, a distinction is made between group I organisms, which are low-risk organisms, and group II organisms, which have higher risk. When group I organisms are used, the authorities are required to be notified, whereas for group II organisms the installations have to be approved.
- Marketing of genetically modified organisms that have been approved for marketing in another EC country, in accordance with regulations laid down by the EC Council of Ministers, requires no approval in Denmark. The Secretary of State for the Environment can forbid the marketing in Denmark, or lay down additional requirements for it, if the marketing conflicts with important environmental or health considerations.

Applications are handled and recommendations given in close cooperation with other departments under the Ministry of the Environment and directorates under other ministries such as the Ministry of Health, the Ministry of Employment and the Ministry of Agriculture. Applications to manufacture GMO's are handled by the National Agency for Environmental Protection.

When deliberate releases are being approved, the National Agency for Environmental Protection sends an advisory report to the Secretary of State for the Environment and the relevant parliamentary committee. The

Secretary of State for the Environment takes the decision on the basis of the report from the National Agency for Environmental Protection and the recommendation of the parliamentary committee.

Regulation in the EC

Two EC directives give guidelines for, respectively, deliberate release and contained use of genetically modified organisms. Anyone wishing to release genetically modified organisms (or a combination of such) for research and development purposes must notify the competent authority in the member country before release takes place. Permission to release GMO's cannot be given without a case-by-case assessment.

The notification must, among other things, contain:

a) a technical description assessing the risks to people and the environment and describing the genetical modification,

b) a declaration carrying an assessment of the probable effects and risks which the genetically modified organism might have for people and the environment.

The competent authority must evaluate the risks attending the deliberate release of GMO's on the basis of the information given by the applicant. If necessary, the authorities may demand further information and can take any samples they consider necessary for control purposes. The authorities in one member country may request information about deliberate releases in another member country. The nation evaluating the terms of the release is, however, under no obligation to respond to comments from the authorities of other nations (Hindar *et al.,* 1990).

As regards marketing, any genetically modified organism or its product that has been approved for marketing inside the EC can, in compliance with rules laid down by the EC Council of Ministers, be marketed in any EC country.

Recommended strategy in the OECD

The OECD published its safety assessments "Recombinant DNA Safety Considerations" in 1986. Attention is focused on safety in relation to industrial manufacture, application in agriculture and release into the environment. The recommendations relating to deliberate release into the environment and use in agriculture are as follows (OECD, 1986, p. 9):

"a) use the existing considerable data on the environmental and human health effects of living organisms to guide risk assessments

b) ensure that recombinant DNA organisms are evaluated for potential risk prior to applications in agriculture and the environment, by means of an independent review of potential risks on a case-by-case basis,

c) conduct the development of recombinant DNA organisms for agricultural or environmental applications in a stepwise fashion, moving, where appropriate, from the laboratory to the growth chamber and greenhouse, to limited field testing and finally, to large-scale field testing,

d) encourage further research to improve the prediction, evaluation, and monitoring of the outcome of applications of recombinant DNA organisms."

The OECD is at present examining how far their guidelines and recommendations are used in the member countries and whether they have been adopted in the laws, regulations and guidelines given by the various countries (OECD, 1990).

4.2 Regulation and management in Norway

Based on White Papers no. 8, 1990-1991 "On biotechnology" and no. 36, 1990-91, "Supplement on biotechnology", a separate joint bill is being prepared to regulate gene and cell technology in Norway. In the supplement, the present government states its view on a set of laws and regulations as follows:

> "Based on its general policy, the government intends to draw up a set of laws and regulations that will ensure the application of gene and cell technology in a manner that is ethical and proper from the viewpoint of society, to safeguard against detrimental effects to health and the environment and to promote sustainable development. In principle, the government advocates that the manufacture and use of genetically modified organisms shall either be notified to the relevant authority or be approved in accordance with agreed regulations. The legislation must in addition open for the possibility of forbidding activity that can involve a significant threat to health and the environment or that raises serious ethical reservations.
>
> The government holds the view that unambiguous regulations must be prepared to regulate, respectively, the contained use and deliberate release of genetically modified organisms.
>
> The government believes that, in principle, genetically modified organisms must not be deliberately released into the environment without special permission, and therefore does not support a general ban with the opportunity of giving dispensation as in the manner stated in White Paper no. 8 (1990-91). Permission can only be given

> when release will be highly advantageous for society in general, at the same time as positive documentation must be supplied proving that the release will not have major detrimental impacts on the environment and health. When such demands are met, permission can be given in individual cases to undertake release in compliance with general regulations, for example of genetically modified crop plants, and when selling products that consist of, or contain, genetically modified organisms".

As a result of a parliamentary debate in June 1991, the management responsibility for modern biotechnology has been divided between the Ministry of Social Affairs and the Ministry of the Environment. The Ministry of Social Affairs has been made responsible for contained use and the Ministry of the Environment for deliberate releases.

The committee considers it propitious that the Ministry of the Environment has been given management responsibility for deliberate and unintentional releases of GMO's.

In most cases, it will be extremely difficult to foresee possible impacts arising from deliberate and unintentional releases of GMO's. It is therefore important that the management authority undertakes a broad and comprehensive ecological evaluation when applications are submitted for the deliberate release of all types of GMO. Consequence reports must be based on the best possible scientific information about the impact of the relevant GMO on the environment.

Since it may be difficult to keep living organisms isolated from the external environment, an evaluation of possible ecological impacts of unintentional releases should also be made available before permission is given for the contained use of GMO's.

5. NEED FOR RESEARCH AND DEVELOPMENT

To be able to provide a good assessment of possible environmental impacts related to deliberate and unintentional releases of GMO's a very broad range of knowledge is required.

Within the fields of molecular biology and gene technology, methods for mapping the genome and the technologically induced changes will be important development areas, along with research into the biochemical and physiological consequences of the genetical change. Methods in gene technology will also be important tools for investigating problem areas in population genetics and ecology.

In population genetics, the question of whether the genetic material from a deliberately released GMO will be able to disperse to free-living populations will be of key importance. This requires knowledge about the genomes themselves, the degree to which viruses and micro-organisms can act as vectors in nature, and the isolating mechanisms at population and species levels.

The chief questions in ecology will be whether GMO's can become established and disperse in the natural environment, and what changes they may bring about to the species and populations comprising the existing communities.

Knowledge about the establishment and dispersal of species and populations can, in traditional terms, be described as autecological and biogeographical problems. The biogeographical elements can also be characterised as colonisation ecology and dispersal ecology and be considered as parts of population dynamics. In the final instance, this, too, will touch upon community ecology, because questions about the extent to which the communities can be colonised will be introduced. The qualitative and quantitative effects of a colonisation and dispersal will need to be based on the population ecology, with emphasis on interspecific interactions such as parasitism, predation and competition, and community ecology with respect to the various stabilising properties held by different communities. The ability to colonise is also part of this complex of problems. Halvorsen & Hartvigsen (1989) give an example of how an introduction problem can be understood in terms of biogeography, population dynamics and community ecology.

Knowledge of the functions and processes that are described above will be important for viruses and all categories of organisms, but in the opinion of the committee, virology, microbiology, botany and invertebrate zoology should be given priority. In virology and microbiology there is a special need to strengthen general ecology and population genetics (particularly knowledge about horizontal gene transfer). In botany, general population dynamics should be strengthened, and in invertebrate zoology special emphasis should be placed on colonisation ecology and dispersal ecology.

From the viewpoint of developing relevant knowledge, it is vitally important that research groups obtain expertise, as well as opportunities in the way of equipment and resources, to carry out a combination of laboratory trials, field trials, field investigations and quantitative models.

To strengthen the kind of general scientific ability described above, it will be natural for the management authorities to seek cooperation with the university and college sector. Since applications to deliberately release GMO's have already been submitted, it is obvious that the management authorities have an immediate need for access to expertise which can investigate and evaluate the scientific aspects relating to such applications. Expertise should therefore be developed further for this purpose within the institute sector. The committee believes the best strategy for this is to supplement the strongly ecology-biased milieu in the institutes with expertise in molecular biology and gene technology. Good contact between the institute and university and college sectors is important.

Hence, the committee believes there to be a great need for research and development in connection with the deliberate and unintentional release of GMO's into the natural environment. One aspect which further emphasises this need is that focusing attention around GMO's will, in the opinion of the committee, also increase the interest for transferring alien populations and species in general, which in turn will lead to an even greater need for the knowledge mentioned above.

6. CONCLUSIONS

The report has shown that insufficient knowledge is available to enable the environmental effects of GMO's to be satisfactorily foreseen. Each individual case must therefore be assessed separately on the basis of data obtained specifically for that investigation.

Various principles and criteria have been worked out in other countries to assess the risks involved in deliberately or unintentionally releasing GMO's into the natural environment. These are chiefly based on general biological and ecological knowledge. The assessments are in most cases reasonable, but it is important to bear in mind that they are no more than that, namely opinions that this or that is likely. The deliberate or unintentional release of GMO's into the natural environment can lead to a large number of different genetical and ecological processes. In many cases, it will be extremely difficult to express any opinion about, and usually impossible to provide any assessment of, the probability of a particular process beginning or not, and if it does what course it will follow. The committee wishes to emphasise this, since the criteria for risk assessment that have been proposed in various contexts are in process of becoming entrenched in the relevant literature as absolute truths. The committee therefore concludes that risk assessment should follow the internationally established form whereby each individual, potential, deliberate release is evaluated separately (case-by-case), through a stepwise procedure (step-by-step), and using as a basis the worst conceivable course. The committee maintains that when these assessments are being made it is not sufficient to draw conclusions on the basis of the phenotype of the GMO; the manipulations that have taken place with the genome must also enter into the assessments.

The applicant for the release should be allowed to carry out the steps in the evaluation that concern the basic biology and the contained trials to describe the physiology and autecology of the GMO. The assessments that have to do with population genetics, population ecology and community ecology should be carried out by independent institutes.

There is a general need for very broadly based research and development work to strengthen our ability to predict what will happen in the event of a deliberate or unintentional release of GMO's into the natural environment. The committee believes that, in this context, research concerned particular with ecology and population genetics in the fields of virology, microbiology, botany and invertebrate zoology should be strengthened at universities and colleges. In the institute sector, the strong ecology milieu should be supplemented by people with expertise in gene technology and molecular biology, to enable the institutes to undertake report preparation and research assignments required by the management sector in association with deliberate or unintentional releases of GMO's and other alien organisms. Because the problems surrounding the deliberate and unintentional release of GMO's into the natural environment have a great deal in common with those found in connection with relocation of populations or species in general, the committee is of the opinion that the same sector of the management authorities should be responsible for both these areas.

The committee believes that, for the moment, it is most convenient to use the relevant international data bases that are available rather than developing one or more Norwegian data bases in this field.

7. GLOSSARY

Autecology: The study of the relationships between single individuals, or the species, and the environment.

Baculoviridae: A virus family that infects invertebrates and that has double-stranded, circular DNA as genetic material. Two groups, polyhedrose and granulose viruses.

Benzene derivatives: A group of substances formed from the hydrocarbon benzene.

Biogeography: The study of the geographical distribution of plants and animals.

Chloroplast: A membrane-enclosed body which is found in plant cells and which performs photosynthetic reactions. It contains chlorophyll which makes plants green.

Chromosome: A DNA chain with a series of genes arranged successively in a row.

Cloning: Techniques used to obtain a collection of genetically identical organisms or cells, or identical DNA molecules.

Coding sequence of DNA: A part of a DNA molecule that codes for one or a few specific genetic products (e.g. proteins).

Dioxin: A group of organic substances containing chlorine. Some are extremely poisonous.

DNA: Abbreviation for deoxyribonucleic acid, which is the chemical compound that is the main constituent of the genetic material in living organisms.

Enzyme: A group of proteins that catalyses and changes the rate of biochemical processes.

Eucaryotic organism: An organism that has cells containing a membrane-bound cell nucleus in which DNA is organised into chromosomes. Higher organisms: protozoa, algae, fungi, mucous mould, plants and animals.

Eutrophication: An increased input of nutrients into a watercourse and its effect.

Foreign protein: The product of a gene from one organism that is expressed in another organism (e.g. a growth hormone from a human expressed in salmon).

Gene: The segment of genetic material (the DNA molecule) that contains information about how a specific protein or parts of a protein are to be constructed.

Gene splicing: Cleaving and sticking together of genes with the aid of molecular biological methods.

Genome: Comprises all the hereditary material in a cell. The genome therefore contains all the information needed to construct an identical, new cell.

Habitat: The area in which a plant or animal lives.

Halogenised hydrocarbons: Hydrocarbons containing one or more halogens (chlorine, bromine, fluorine, etc.).

Haemoglobin: An oxygen-binding protein in red blood cells. Transports oxygen in the blood of higher animals.

Herbicides: Chemical substances to control plants, used against plants that are considered harmful for agriculture, forestry and horticulture, etc.

Hormones: Chemical messengers that are manufactured and secreted in small quantities in special tissues and that regulate biological processes elsewhere in the organism.

Hybrid: The progeny of a cross, or resulting from hybrid cell techniques between two different species of plant or animal, respectively.

Immunology: The study of the ability of an organism to withstand infections.

Invertebrates: All animals lacking a backbone, i.e. all groups of animals apart from the vertebrates.

Horizontal gene transfer: Transfer of genes without sexual reproduction between species.

Leghaemoglobin: Oxygen-binding protein found in the nitrogen-synthesising root nodules of leguminous plants.

Marker gene: A gene that codes for a very specific property and is inserted as a marker into an organism or cell attached to the gene that needs to be expressed. It codes for a property that is easily registered or recognised (e.g. resistance to antibiotics).

Micro-injection: A technique used to inject naked DNA directly into a cell or cell nucleus.

Mitochondria: Membrane-enclosed bodies in the cells of eucaryotic organisms. They contain a tiny, circular DNA and are important for the energy conversion of the cells.

Mutation: A random change in the DNA molecule. It can occur spontaneously, under the influence of UV radiation or through the use of certain chemicals.

Nematode: Roundworm. Often a parasite in plants and animals.

Parasite: An organism which for the whole or part of its life lives in or on an organism of another species (the host). It obtains nourishment from the host and can cause considerable damage.

Pathogen: A disease-causing micro-organism.

PCB: Polychlorinated biphenyl. A group of halogenised hydrocarbons which are difficult to break down in nature.

Pesticides: Chemical substances used against pests and other harmful organisms. Include fungicides, herbicides and insecticides.

Phenotype: The visible or measurable physical and biochemical characters of an organism. A result of interaction between the genotype and the environment.

Plasmid: A DNA molecule in bacteria and some other organisms, which is able to self-replicate. Exists outside and partly independently of the chromosomes. Can code for genetical products that affect the characteristics of the host cell.

Polyploid: An organism that has more than two chromosome sets per somatic cell.

Population: A group of potentially interbreeding individuals which co-exist in time and space.

Population genetics: The study of the genetic composition of a population and the factors affecting it.

Procaryotic organism: A single-celled organism that lacks a membrane-bound cell nucleus or other membrane-bound structures, such as mitochondria and chloroplasts. Chiefly bacteria and cyanobacteria (blue-green algae).

Proto-oncogene: A normally-celled gene whose genetic product affects the growth of the cell. If the gene is altered (e.g. by mutation) or over-expressed, the cell can be transformed into a cancer cell.

Protoplast: A plant cell that has had its cell wall removed.

Recessive mutation: A mutation that is not reflected in the phenotype.

Recombinant DNA: A DNA molecule produced by the combining or binding together of two sequences from two or more separate DNA molecules. Recombinant DNA can occur naturally, but the term is chiefly used about DNA that is constructed by genetical engineering methods (e.g. human DNA inserted into a plasmid).

Regulatory sequence of DNA: A part of the DNA molecule that determines whether, and to what extent, the coding segments of the DNA molecule are to be expressed.

Resistance: Capacity to withstand. Often used about the degree of capacity to withstand diseases.

Ribosome: A macro-molecule in cells. It is constructed of RNA and proteins. The site where amino acids are synthesised to form proteins.

RNA: Abbreviation for ribonucleic acid. Translates, for example, information from the genes to ribosomes outside the cell nucleus where amino acids are synthesised to form proteins. RNA may also be the hereditary material in certain viruses.

Selection pressure: The effect of any environmental factor that results in natural selection, e.g. antibiotics in the environment can lead to selection pressure for antibiotic resistance in bacteria.

Sequencing: Mapping the order of bases in DNA molecules or the order of amino acids in protein molecules.

Symbiont: One of the partners in a symbiosis, which is a mutual dependency relationship between two organisms belonging to different species.

Toxin: Any poisonous substance from a micro-organism, plant or animal.

Toxin gene: A gene which codes for the formation of a toxin.

Transcriptase: A group of enzymes which catalyses the transcription.

Transcription: The process whereby a DNA sequence is copied with the help of an RNA polymerase enzyme, forming a complementary RNA copy of the DNA sequence.

Transduction: A natural process whereby genes are transferred between bacteria via bacteriophages.

Transformation: When bacteria take up naked DNA from the environment.

Translocation: Movement of genes or parts of chromosomes from one chromosome to another.

Transposon: A DNA sequence that can move within the genome. Larger bacterium transposons can, for example, move genes to provide antibiotic resistance.

Vaccinia virus: Belongs to the Poxviridae family which has double-stranded DNA. Often used as a vector for vaccine-associated genes and other genes.

Vector: A carrier of DNA from a donor cell or organism to a recipient. The vector is often a plasmid or parts of a bacteriophage, and is used in genetical engineering to transfer genes.

Virology: The study of viruses.

Virus: A non-cellular particle consisting of a DNA or RNA molecule surrounded by a protein shell. It can only reproduce in living cells.

8. REFERENCES

Allee, W. C., Emerson, A. E., Park, O., Park, T. & Schmidt, K. 1949. Principles of animal ecology. Saunders, Philadelphia.

Baker, H. G. & Stebbins, G. L., eds. 1965. The genetics of colonizing species. Academic Press, New York.

Baltz, D. M. 1991. Introduced fishes in marine systems and inland seas. Biol. Conserv., 56: 151-177.

Bergh, Ø., Børsheim, K. Y., Bratbak, G. & Heldal, M. 1989. High abundance of viruses found in aquatic environments. Nature (Lond.) 340: 467-468.

Bishop, D. H. L. 1988. The release into the environment of genetically engineered viruses, vaccines and viral pesticides. Special combined issue; Trends Biotech. 6 & Trends Ecol. Evol. 3: 12-15.

Bye, K., Halvorsen, O., & Nilssen, K. 1987. Immigration and regional distribution of abomasal nematodes of Svalbard reindeer. J. Biogeography 14: 451-458.

Crawley, M. J. 1988. Meeting report: COGENE/SCOPE at Lake Como. Special combined issue; Trends Biotech. 6 & Trends Ecol. Evol. 3: 2-3.

Davies, J. 1988. Genetic engineering: Processes and products. Special combined issue; Trends Biotech. 6 & Trends Ecol. Evol. 3: 7-11.

Drake, J. A., Mooney, H. A., di Castri, F., Groves, R. H., Kruger, F. J., Rejmánek, M. & Williamson, M., eds. 1989. Biological invasions: A global perspective. SCOPE 37, Wiley, Chichester, U.K.

Ebenhard, T., 1988. Introduced birds and mammals and their ecological effects. Viltrevy, 13 (4): 5-107.

Egglestone, P. 1991. The control of insect-borne disease through recombinant DNA technology. Heredity 66: 161-172.

Ellstrand, N. C. 1988. Pollen as a vehicle for the escape of engineered genes? Special combined issue; Trends Biotech. 6 & Trends Ecol. Evol. 3: 30-32.

Elven, R., Fremstad, E. & Sandved, M. 1991. Genetiske risikoer for norske villplanter. NINA Oppdragsmelding 73: 1-39.

Furner, I. J., Huffman, G. J., Amasino, R. M., Garfinkel, D. J., Gordon, M. P. & Nester, E. W., 1986. An agrobacterium transformation in the evolution of the genus *Nicotiana*. Nature (Lond.) 319: 422-427.

Gaertner, F. & Kim, L. 1988. Current applied recombinant DNA projects. Special combined issue; Trends Biotech. 6 & Trends Ecol. Evol. 3: 4-7.

Gaugler, R. 1987. Entomogenous nematodes and their prospects for genetic improvement. In K. Maramorosch, ed., Biotechnology in invertebrate pathology and cell culture. Academic Press, 457-483.

Gibbs, J. N., Cooper, I. P., & MacKler, B. F., eds. 1987. Biotechnology & the environment: International regulation. Macmillan.

Goksøyr, J. & Sørheim, R. 1991. Utsetting av genmodifiserte organismer. Kontrakt nr. BTEK 2, Direktoratet for Naturforvaltning.

Griffith, B., Scott, J. M., Carpenter, J. W. & Reed, C. 1989. Translocation as a species conservation tool: Status and strategy. Science (Wash.) 245: 477-480.

Groves, R. H. & Burdon, J. J., eds. 1986. Ecology of biological invasions. Cambridge University Press, Cambridge, U. K.

Halvorsen, O., & Hartvigsen, R., 1989. A review of the biogeography and epidemiology of *Gyrodactylus salaris*. NINA Utredning 2: 1-41.

Harlan, J. R. 1975. Crops and man. American Society of Agronomy, Madison, WI.

Hartl, D. L., Dykhuizen, D. E., Miller, R. D., Green, L. & DeFramond, J. 1983. Transposable element IS50 improves growth rate of *E. coli* cells without transposition. Cell 35: 503-510.

Hindar, K. & Bakke, Ø., 1991. Miljøeffekter av utsetting av genmodifiserte organismer. NINA Oppdragsmelding 72: 1-77.

Hindar, K., Rosendal, G. K. & Trønnes, H. N. 1990. Bioteknologi og norsk tilpasning til EFs indre marked. NINA Utredning 8: 1-51.

Hindar, K., Ryman, N. & Utter, F. 1991. Genetic effects of cultured fish on natural fish populations. Can. J. Fish. Aquat. Sci. 48: 945-957.

Howarth, F. G. 1991. Environmental impacts of classical biological control. Ann. Rev. Entomol., 36: 485-509.

Kaplan, C. 1989. Vaccinia virus: a suitable vehicle for recombinant vaccines? Arch. Virol. 106: 127-140.

Keeler, K. H. 1985. Implications of weed genetics and ecology for the deliberate release of genetically engineered crop plants. Recomb. DNA Tech. Bull. 8: 165-172.

Kjelleberg, S. & Fagerström, T., eds. 1990. Ekologiska risker med spridning av transgena organismer: Underlag til forskningsprogram. Rapport 3689, Statens naturvårdsverk, Solna.

Lensky, R. E. & Nguyen, T. T. 1988. Stability of recombinant DNA and its effects on fitness. Special combined issue; Trends Biotech. 6 & Trends Ecol. Evol. 3: 18-20.

Mehl, R. 1991. Biologisk bekjempelse av skadedyr i Norge. In Vedlegg til rapporten: Økologisk risiko ved utsetting av genmodifiserte organismer i naturen. DN-notat nr. 10.

Miller, D. J. 1989. Introductions and extinction of fish in the African Great Lakes. Trends Ecol. Evol., 4: 56-9.

Miller, R. V. 1988. Potential for transfer and establishment of engineered genetic sequences. Special combined issue; Trends Biotech. 6 & Trends Ecol. Evol. 3: 23-27.

NOU. 1990. Moderne bioteknologi, Sikkerhet, helse og miljø. Norges offentlige utredninger 1990/1.

OECD. 1986. Recombinant DNA safety considerations. Organisation for Economic Co-operation and Development, Paris.

OECD. 1990. International survey on biotechnology use and regulations. ENV/BIO/89.1, OECD Environment Directorate, Paris.

OTA, 1988. New developments in biotechnology (3). Field testing engineered organisms: genetic and ecological issues. Office of Technology Assessment, US Congress, Washington D.C.

Paigen, K. 1986. Gene regulation and its role in evolutionary processes. In: S. Karlin & E. Nevo, red.: Evolutionary process and theory. Academic Press, Orlando.

Pimentel, D. 1986. Biological invasions of plants and animals in agriculture and forestry. In H. A. Mooney & J. A. Drake, eds. Ecology of biological invasions of North America and Hawaii. Ecological studies 58: 149-162, Springer-Verlag, New York.

Rognli, O. A. 1991. Genmodifiserte planter. In Vedlegg til rapporten; Økologisk risiko ved utsetting av genmodifiserte organismer i naturen. DN-notat nr. 10.

Roughgarden, J. 1986. Predicting invasions and rates of spread. In H. A. Mooney & J. A. Drake eds., Ecology of biological invasions of North America and Hawaii. Ecological Studies 58: 179-188, Springer-Verlag, New York.

Royal Commission. 1989. The release of genetically engineered organisms to the environment. Royal Commission on Environmental Pollution, 13th report, HMSO, London, U.K.

Ryman, N. & Utter, F., eds. 1987. Population genetics and fishery management. University of Washington Press, Seattle, WA.

Rørslett, B. & Berge, D. 1983. Vasspest. In Berge, D. eds., Tyrifjorden, 95-103. Sluttrapport fra Tyrifjordundersøkelsen, NIVA, Oslo.

Simberloff, D. 1991. Keystone species and community effects of biological introductions. In L. R. Ginzburg, eds., Assessing ecological risk of biotechnology, 1-19, Butterworth-Heinemann, Boston.

Simonsen, L. & Levin, B. R. 1988. Evaluating the risk of releasing genetically engineered organisms. Special combined issue; Trends Biotech. 6 & Trends Ecol. Evol. 3: 27-30.

Singer, M. & Berg, P. 1991. Genes and Genomes. Blackwell Scientific Publications.

Skellam, J. G. 1951. Random dispersal in theoretical populations. Biometrika 38: 196-218.

Stearns, S., Meyer, J. & Shykoff, J. 1988. Meeting report: COGENE 88 at Basel. Special combined issue; Trends Biotech. 6 & Trends Ecol. Evol. 3: 2-3.

St. meld. nr. 8 (1990-91). Om Bioteknologi. Miljøverndepartementet, Norge.

St. meld. nr. 36 (1990-91). Tilleggsmelding om bioteknologi. Miljøverndepartementet, Norge.

TeknologiNævnet. 1990. Økologiske forskningsbehov i lyset af den bioteknologiske udvikling. TeknologiNævnets rapporter 1990/1, København.

Templeton, A. 1986. Coadaptation and outbreeding depression. In M. E. Soulé, red., Conservation biology. Sinauer, Sunderland, MA. : 105-116.

Thomson, B. J., Efstathiou, S. & Honess, R. W. 1991. Acquisition of the human adenoassociated virus type-2 rep gene by human herpes virus type-6. Nature (Lond.) 351: 78-80.

Tiedje, J. M., Colwell, R. K., Grossman, Y. L, Hodson, R. E., Lenski, R. E., Mack, R. N. & Regal, P. J. 1989. The planned introduction of genetically engineered organisms: Ecological considerations and recommendations. Ecology 70: 298-315.

United States General Accounting Office, 1988. Biotechnology: Risk management, GAO/RCD-88-27.

Yoccoz, G. N., Steen, H., & Ims, R. A. 1990. Østmarkmus: En ny pattedyrart for Svalbard. Fauna, 43 (1): 36-42.

Yoder, O. C., Weltring, K., Turgeon, B. G., Garber, R. C. & Vanetten, H. D. 1987. Prospects for development of molecular technology for fungal insect pathogens. In K. Maramorosch, ed., Biotechnology in invertebrate pathology and cell culture. Academic Press, 197-218.

Williamson, M. 1989. Potential effects of recombinant DNA organisms on ecosystems and their components. Special combined issue; Trends Biotech. 6 & Trends Ecol. Evol. 3: 32-35.

Williamson, M. H. & Brown, K. C. 1986. The analysis and modelling of British invasions. Phil. Trans. R. Soc. London Ser. B 314: 505-522.

Williamson, M., Perrins, J. & Fitter, A. 1990. Releasing genetically engineered plants: present proposals and possible hazards. Trends Ecol. Evol., 5: 417-419.

9. ENCLOSURE

PROGRAMME

WORKSHOP ON THE CONSEQUENCES OF THE DELIBERATE RELEASE OF GENETICALLY MODIFIED ORGANISMS INTO THE NATURAL ENVIRONMENT
RICA HELL HOTEL
21-23 MAY 1991

Tuesday 21/5

12.00 Arrival and lunch

13.00 Opening by Astrid Langvatn, assistant director of DN, and a representative of the committee.

13.30 Effects of increased gene flow between populations. Lecture by Kjetil Hindar (NINA).

14.10 Should the phenotype be the only basis for risk evaluation? Lecture by Bjørn Lindqvist.

14.50 Division into groups.

Group 1. Gene technology: Can gene technologists predict where the gene will end up in the genome and what effect it will have?

Group 2. Population genetics: Which genetically modified organisms can exchange genes with wild populations and closely related species?

Group 3. Ecology: What can we predict about the environmental effects of genetically modified organisms?

15.10 Coffee

15.40 Group discussions

17.30 Summing up of group discussions in plenary

18.30 Closing

19.00 Dinner

Wednesday 22/5

09.00 Division into groups according to specialist fields, to evaluate the report.

Group 1. Micro-organisms and viruses
Group 2. Plants
Group 3. Animals

11.00 Coffee

11.30 Summing up of group discussion in plenary.

12.30 Lunch

13.30 Invasion: some ecological thoughts around the environmental impact of releasing genetically modified organisms. Nils Chr. Stenseth (Univ. of Oslo).

14.00 Plenary discussion about ecological effects.

14.30 Questions in plenary. Are Norwegian ecosystems vulnerable to invasion by GMO's? Discussion.

15.30 Coffee

16.00 Group discussion around the question: Where does the boundary go between contained use and release? (Group division as during the report discussion).

17.00 Experience of regulation of biotechnology in Denmark. Jens Chr. Pedersen (Environmental Survey of Denmark)

17.20 Plenary discussion. Regulation of biotechnology.

18.00 Closing

19.00 Dinner

Thursday 23/5

09.00 Division into groups to discuss what research is required in Norway (same division as during the report discussion).

10.30 Coffee

11.00 Summing up, discussion in plenary

12.00 Closing and lunch

Participants at the workshop on the releasing of genetically modified organisms, 21-23 May 1991, at Rica Hell Hotel.

Reidar Almås,
Centre for Village Research,
University of Trondheim,
N-7055 Dragvoll

Øyvind Bakke,
Norwegian Institute for Nature
Research,
Tungasletta 2,
N-7005 Trondheim

Atle Bones,
Institute of
Biotechnology/UNIGEN,
Norwegian Institute of
Technology,
University of Trondheim,
N-7034 Trondheim

Heidi Bente Hoel Draget,
Directorate for Nature
Management,
Tungasletta 2,
N-7005 Trondheim

Bodil Eggen,
State Pollution Control Authority,
P.B. 8100 Dep.,
N-0032 Oslo 1

Steinar Engen,
Institute of Mathematics and
Statistics,
University of Trondheim,
N-7055 Dragvoll

Jostein Goksøyr,
Institute of Microbiology and
Plant Physiology,
University of Bergen,
Jahnebakken 5,
N-5007 Bergen

Kjetil Hindar,
Norwegian Institute of Nature
Research, Tungasletta 2,
N-7005 Trondheim

Jan Husby,
Directorate for Nature
Management,
Tungasletta 2,
N-7005 Trondheim

Terje Johansen,
Institute of Medical
Biology,
University of Tromsø,
P.B. 635,
N-9001 Tromsø.

Bjørn H. Lindqvist,
Centre for Biotechnology in
Oslo,
P.B. 1125 Blindern,
N-0371 Oslo 3.

Ivar Lossius,
Department of Research
Administration,
University of Bergen,
Museumsplass 2,
N-5020 Bergen.

Morten Laake,
Norwegian Institute for
Water Research,
P.B. 69 Korsvoll,
N-0808 Oslo 8.

Reidar Mehl,
National Institute of Public Health,
Geitemyrsvn. 75,
N-0462 Oslo 4.

Jarle Mork,
Trondheim Biological Station,
University of Trondheim,
N-7055 Dragvoll.

Jens Chr. Pedersen,
Department of Marine
Environment and
Microbiology, Mørkhøj,
Environmental Survey of
Denmark, Mørkhøj Bygade 19,
Søborg
D-2860 Denmark.

Sissel Rogne,
Institute of Animal Husbandry,
Agricultural University of
Norway,
P.B. 25,
N-1432 Ås.

Odd Arne Rognli,
Institute of Biotechnology,
Genetics and Plant Breeding,
Agricultural University of
Norway,
P.B. 40,
N-1432 Ås.

Nils Chr. Stenseth,
Department of Zoology, Institute
of Biology,
University of Oslo,
P.B. 1050 Blindern
N-0136 Oslo 3.

Yngve Svarte,
Directorate for Nature
Management,
Tungasletta 2,
N-7005 Trondheim.

Bernt-Erik Sæther
Norwegian Institute for Nature
Research,
Tungasletta 2,
N-7005 Trondheim.

Terje Traavik,
Institute of Medical Biology,
University of Tromsø,
P.B. 635,
N-9001 Tromsø.

Svein Valla,
Institute of Biotechnology,
UNIGEN, Norwegian Institute of
Technology
University of Trondheim,
N-7034, Trondheim.

Reidun Aalen,
Agricultural Research Council of
Norway's Laboratory of Plant and
Molecular Biology,
Agricultural University of
Norway,
P.B. 51,
N-1432 Ås.

Price NOK 30,-

DIRECTORATE FOR NATURE MANAGEMENT
Tungasletta 2, N-7005 Trondheim. Tel. + 47 58 05 00

ISSN 0801-6119
TE-538